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MELVILLE T. COOK, Editor.

A New Mechanical Method for Artificially Transmitting Sugar-Cane Mosaic

By

FRANCISCO SEIN, JR.

The Effect of Some Mosaic Diseases on Cell Structure and on the Chloroplasts

By

MELVILLE T. COOK

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A NEW MECHANICAL METHOD FOR ARTIFICIALLY TRANSMITTING SUGAR-CANE MOSAIC

By FRANCISCO SEÍN, JR.

The study of sugar-cane mosaic transmission and its insect vectors started at the Insular Experiment Station in 1918 by Smyth and carried on by Wolcott and Chardon has been continued by the author since 1922 up to the present date.

For the purpose of obtaining data on how the virus of sugar-cane mosaic is introduced by the insect vector into the tissues of the healthy plant, a new method of artificial inoculation has been devised which due to its simplicity and efficiency has proved very helpful.

A very few notes on this new method and its operation were advanced in the Report of the Division of Entomology, Annual Report of the Insular Experiment Station of Porto Rico, 1927–1928. The Director of the Insular Station, Mr. R. Fernández García, has informed us that he reported it at the Third Congress of the International Society of Sugar Cane Technologists held in Java in June 1929.

Mr. Authur H. Rosendfeld, in the February, 1930, number of the *International Sugar Journal,* commenting on the Java Meeting of the International Society of Sugar Cane Technologists when refering to the paper on "Mechanical Transmission of Mosaic" by Miss G. Wilbrink writes that:

> "During the discussion of this paper it was brought out that Mr. F. Seín had developed a simple method for the mechanical transmission of sugar-cane mosaic at the Porto Rico Insular Experiment Station for use in the testing out of varieties as regards natural resistance to the disease. The device seems to be a very simple one, consisting of a number of pin points at the end of a small handle, and, with this device, Mr. Seín has succeeded in a great many cases in transmitting mosaic from an affected plant to an unaffected one simple by pricking the leaves of the affected plant first and then proceeding to do the same thing with the leaves of the healthy plant."

As stated above by the author, his purpose in devising a new method of artificial inoculation was the study of how the causative principle of the disease is introduced into the tissues by the insect

vector. The practical application of the new method in sugar-cane breeding work and in the study of the disease itself came up as secondary to the entomological problem which was contemplated. The reader will also become aware that the description of the method, the instrument used and the technique followed by the author are a little different from the description Mr. Rosenfeld has kindly advanced.

Since various mechanical methods for the artificial transmission of sugar-cane mosaic have been devised by previous investigators, and since there are divergent opinions on their efficiency, a review of the experimental work on this subject is desirable.

REVIEW OF THE LITERATURE ON THE TRANSMISSION OF SUGAR-CANE MOSAIC BY MECHANICAL MEANS

The mosaic disease of sugar cane was first reported from Java in 1890 by Dutch investigators who became convinced after unsuccessful attempts to transmit it artificially, that they were dealing with a bud variation rather than a disease. Kamerling (**9**), 1890, in Java reported successful infections obtained, we judge, by rubbing the lacerated leaves of healthy cane plants with the juice pressed out of mosaic plants. In the same publication Kamerling admits the occurrence of disease in his control plants and later Dutch investigators discredited his experiments. Kobus (**10**), van der Stok (**19**) and Wilbrink and Ledeboer (**21**), failed to produce the disease repeating Kamerling's experiments.

In America, Stevenson (**18**) was the first to work on sugar-cane mosaic. He began his studies at the Insular Experiment Station at Río Piedras, Porto Rico, in 1916, and continued them until 1919 but failed in all his attempts to transmit the disease artificially. Stevenson attempted to produce the disease by inoculating cane with pure cultures of several kinds of fungi; by hypodermic injections of the juices extracted from mosaic cane tissues; by placing the juice in holes made in the stalks; by inserting small bits of diseased tissue into various parts of healthy canes; and by rubbing the growing tips of the stalks after a diseased tip had been crushed in the fingers.

Tower (**20**), 1919, of the Federal Experiment Station, Mayagüez, P. R., failed to transmit sugar-cane mosaic by crushing and rubbing mealy bugs from diseased cane on developing buds and shoots of healthy canes, and by forcing the crushed bugs into punctures made in the buds and shoots.

Earle (**6**), 1920, at the Insular Experiment Station, P. R., performed a very large number of experiments in which he exhausted his ingenuity in the attempt to transmit the disease artificially and to reproduce the work of the unknown, but suspected insect vectors. The experiments were performed in the open and thus there was always the possibility of error. The percentage of infections in his inoculated canes was exceedingly low but Earle believed that a portion at least of these cases were caused by artificial inoculation. But he comments: "The fact remains, however, that the successes were much less frequent than the failures, that the best results could not always be duplicated and that the successful transfer of the disease is dependent on some factor or factors as yet absolutely undiscovered." Earle tried the following methods: rubbing or otherwise lacerating healthy leaves with diseased tissues; binding pieces of diseased tissue in contact with cut surfaces of healthy stalks; dropping bits of diseased tissue into the unrolled terminal leaf spindle and injections of the extracted juices by means of a hypodermic syringe. He also made a very interesting experiment in which a hypodermic needle was thrust into the soft tissue near the terminal bud of a diseased cane and then immediately inserted near the base of the unrolled leaf spindle of a healthy cane plant. There was no transmission in fifty attempts. This experiment although unsucessful is of interest to us because it is a departure from the attempt to imitate the work of a sucking insect. In a further experiment, where the attempt was to imitate the insect by extracting the juices from mosaic tissues and injecting them into healthy plants without exposure to the air, Earle was more successful. As this method was used later by Brandes and Bruner it is desirable to explain that upon the suggestion made by Mr. F. A. López Domínguez, Prof. Earle and Mr. E. D. Colón extracted the juice from the tender parts of mosaic cane plants by grinding with a pestle under a layer of mineral oil in a porcelain mortar. The juice was then taken up with a syringe, the needle of which was inserted through the oil layer, and injected immediately into healthy cane plants. With this method Earle was able to obtain five infections out of ten inoculated plants. On repeating the experiment two successive times, however, he was unable to obtain a single infection.

Matz (**13**), 1920, working also at the Insular Experiment Station, P. R., carried on an extensive series of complicated experiments in the endeavor to transmit sugar-cane mosaic artificially. His percentage of infection was extremely low and he concluded that "the exact method to insure takes is not known as yet". Matz tried the fol-

lowing methods: healthy and mosaic cane plants set in the same pots; healthy and diseased seed pieces split in half, a diseased half and a healthy half fastened together and then planted; healthy seed pieces watered with water in which diseased cane was allowed to stay for some time; and buds from healthy seed pieces inserted in diseased seed pieces and vice-versa, but no transfer of the disease took place.

Matz performed several experiments with juice pressed out of mosaic cane tissues exposed to the air. Healthy cane stalks in three pots inside the greenhouse were cut back leaving stumps about four inches above ground. There were one or more shoots about six inches high emerging from the base of the stumps. The juice was injected with a hypodermic needle into the stumps near the surface of the ground. The three shoots in one pot developed mosaic. The rest remained healthy. The experiment was repeated with twenty plants leaving twenty uninoculated as check. Two of the inoculated developed mosaic.

In another experiment ten cane plants were cut back, only a little above the growing point, five were inoculated in the cut surface of the top by injecting diseased juice with a hypodermic needle and five were left as checks. All remained healthy.

Next, twenty-five healthy stools in the greenhouse were cut back as in previous experiments and eight were inoculated with diseased juice and in addition, pieces of diseased cane were forced into small holes in the stems. All twenty-five plants remained healthy and *one of the checks developed the disease.*

The successful inoculations obtained in the first experiments were performed on cane more mature than that used in the last. To test this point eighteen seed pieces of mature Cristalina cane were cut to one or two eyes, twelve were inoculated near the base of the bud by boring a hole into the seed piece three-fourths inch deep and directly into it was pressed juice from diseased cane and six were inoculated in the same way with healthy cane juice. At the same time 35 Cristalina stools in a field that had just been cut were inoculated with juice in the stubble near the bases of sprouting buds. In both of these last two experiments not a single positive case developed. Matz remarks: "It was thought that by bringing in contact the cut ends of the vascular systems of diseased and healthy cane a transmission of the disease might take place. But no infection occurred". This is very interesting when compared with what Bonazzi reported in Cuba and Cook in Porto Rico later on.

The case of infection in his checks that we have specified is one of the several reported by Matz.

Smyth (**17**), 1920, at the Insular Station also performed twenty-seven experiments which consisted in crushing the juices of mosaic cane tissues into the leaf tissues of healthy cane plants, using very young, vigorous plants, and seventeen experiments which were attempts to transmit the disease by spreading quantities of finely-cut-up diseased tissue, either in juicy or dry condition, over the healthy plants and over the soil immediately surrounding them. All these forty-four tests gave negative results.

Lyon (**12**), 1921, in Hawaii, conducted numerous experiments in attempts to convey the disease by artificial means. The media most employed were juices extracted by pressure from various parts of affected canes, but more particularly from tissues adjacent to the growing point of the stem. Inoculation was attempted by applying these juices externally to all parts, and introducing them internally at various points in the stem, eyes and spindle with a needle-syringe. In no case did the disease appear on a high percentage of treated canes or canes from treated cuttings, and in every experiment it appeared on such a number of shoots in the checks as to invalidate the evidence of artificial infection. Most of these experiments were conducted under open field conditions and in a restricted area where the disease was at the time present in nearby canes. In the few experiments where canes grown in tubs and carefully isolated in a glass house were employed, only negative results were obtained.

Brandes (**1**), in 1920, performed his well-known experiments at Washington, D. C., in which he showed by eleven successful inoculations that mosaic could be transmitted artificially but under great difficulties. Bruner (**4**) remarks: "since Brandes' limited experiments were not repeated under the same or more natural conditions it is not sure that the same results could be duplicated."

In Brandes' experiments, "virus" was obtained for artificial inoculation by two methods. Cell sap from young leaves, designated as virus No. 1, was obtained by grinding the young, tightly rolled leaves of diseased Rayada cane in a food chopper and straining through several thicknesses of cheese cloth. It was used undiluted for inoculation immediately after being prepared. Virus No. 2, consisted of cane juice from the youngest joints, including the growing point. To prevent oxidation this was pressed out under a mineral oil (Nujol) in a specially designed press. This also was used undiluted as soon as prepared. Inoculations were made in a compartment of a fumigated greenhouse separated from all diseased material

and protected by every means from accidental infection. The results of these inoculations show that where the virus No. 1 was used, one plant out of ten developed mosaic when the "youngest leaves were inoculated by numerous needle pricks." No more explanation as to Brandes' technique is given, but presumably the virus was smeared on the leaf surface and pricked in with the needle. Where virus No. 2 (extracted under oil) was used, presumably in identical manner, none of the ten plants developed the disease. Where ten plants were inoculated by injecting ½ cc., of virus No. 1 into the growing point with a hypodermic syringe, two out of ten developed mosaic, and where virus No. 2 was used in the same manner, eight out of ten became diseased. Brandes infers from these results that the sugarcane mosaic virus is highly infectious only when exacting demands in the matter of favorable conditions are satisfied. It is considered as proved, however, that the cell sap of diseased plants is infectious when introduced in the proper manner.

Bruner, (**4**), 1922, in Cuba published the results of his transmission expermients conducted, as he says "since we consider it necessary to be able to transmit the disease artificially before we can study it more thoroughly." Bruner repeated experiments made by Earle and Brandes. Twelve cane plants were inoculated by injecting juice from mosaic cane tissues extracted under oil. Castor oil was used and a few cubic centimeters of distilled water were placed in the bottom of the press before adding the oil: all inoculated plants remained healthy. Thirteen cane shoots were inoculated with juice obtained as in the previous experiment except that Nujol was used instead of castor oil: two developed the disease, or fifteen per cent. Ten plants were inoculated with juice obtained as in the last experiment but three injections performed in each plant: three of the inoculated plants became mosaic, or thirty per cent.

Thirty plants were divided in two groups and the plants in each group given two injections. Fifteen were inoculated immediately after extracting the juice exposed to the air: three became mosaic. Fifteen others were inoculated immediately after extracting the juice under Nujol oil: three developed the disease. Checks were kept in all these experiments. In no case did mosaic appear in the checks. From his experiments Bruner concludes that undoubtedly sugarcane mosaic can be transmitted artificially but that there is yet to be found a method which will produce a constant high percentage of infections since there is some factor that influences them which is not known.

Bruner performed also an experiment similar to the one performed

by Earle, thrusting rapidly a fine hypodermic needle through a freshly cut mosaic cane leaf so that it would penetrate directly into the central nerve (or near to it) of the healthy leaf against which it was held in contact. This was done with the purpose of introducing the virus of the disease into the tissues of the healthy plant with the minimum waste of time and exposure to the air. In this manner, three leaves were inoculated in each plant; one tender, one mature and one old leaf, making three perforations in each leaf. Check plants were identically treated omitting only the diseased leaf.

Kind and condition of the plant: The plants inoculated were of the Cristalina variety, old ratoons about 1½ meters high in a field where infections of cane mosaic had never been observed.

Number of inoculated plants: 100 shoots in 23 stools
Date on which inoculated: December 6, 1920
No indications of infection: January 7, 1921
March 9, 1921:
Stool No. 4 out of 4 shoots, 3 are mosaic
Stool No. 5 out of 4 soots, 1 is mosaic
Stool No. 18 out of 10 shoots, 4 are mosaic

All the check plants remained healthy. As further precaution against error, all the other stools in the field were examined and no infection was found in them. Bruner concludes that the eight plants inoculated became diseased as a result of the introduction into them of the infectious agent of mosaic when the perforations were made with the needle. Bruner, however, does not take into consideration that the virus of sugar-cane mosaic moves from one shoot to another in the same stool. This has been reported by Earle (**6**) and by Menéndez Ramos (**16**) and confirmed by the writer in unpublished experiments. Thus, there may have been only one successful inoculation in each stool.

Kunkel (**11**), in Hawaii, reports an experiment on mosaic transmission performed December 8, 1921. Undiluted juice pressed from the leaves and upper joints of diseased Lahaina cane was rubbed into wounds made on the leaves of six healthy Striped Tip cane plants. The leaves were wounded by crushing them between finger and thumb. The wounded tissue was inoculated by rubbing it with a small piece of absorbent cotton saturated with the diseased juice. Since no infections were obtained the inoculations were repeated December 26 and January 4, 1922. On April 7, five of the six plants inoculated had mosaic.

Fawcett (**7**), 1925, in Argentina has reported some very interesting experiments. His first attempt to transmit sugar-cane mosaic

by wetting the leaves of healthy cane shoots fifty centimeters high with virulent cane juices failed, but upon repeating the experiment with five cane shoots ten to twenty centimeters high they all developed mosaic. At the end of three weeks the inoculated shoots showed well-marked symptoms of mosaic while eleven similar shoots growing close to them and left as checks remained healthy. Fawcett's paper is published in the Spanish translation and it is not clear whether the virulent cane juices were merely placed on the leaves or whether pieces of cotton wet with the juices were attached to the leaves. In either case, however, no perforations were made or the leaf surface broken up in any way. As an explanation, Fawcett says:

> "The other well-known disease: tobacco mosaic, is transmitted easily by contact. The application of sap from diseased plants to healthy plants is sufficient to produce infection. To determine whether sugar-cane mosaic can be transmitted in this manner, juices were extracted from mosaic cane leaves by cutting them into bits, adding a little distilled water, grinding and extracting by pressure."

It is evident that Fawcett attempted to transmit sugar-cane mosaic like tobacco mosaic and it is most remarkable that he was able to obtain 100 per cent infection with shoots ten to twenty centimeters high after having failed utterly with shoots fifty centimeters high. No one else has being able to transmit sugar-cane mosaic in this manner.

In another experiment, Fawcett inoculated the virulent juices into the tender leaves in the spindle a short distance above the growing point. He inoculated eleven shoots 10 to 20 centimeters high leaving twelve similar shoots untreated as checks. Between one and two cubic centimeters of virulent juices were inoculated into each shoot. Forty days later, seven out of the eleven inoculated shoots had developed mosaic and all the rest remained healthy.

On repeating the experiment, Fawcett inoculated five small shoots leaving similar shoots as checks. Twenty-four days later three out of the five inoculated shoots had developed mosaic. The others remained healthy.

It is very surprising to see that Fawcett should have obtained a higher percentage of infections when the virus was merely placed on the leaves rather than when it was injected. Fawcett, however, does not consider it significant and he adds:

> "The effect of injections is essentially the same as that of the application to the leaves differing only in that the virus is placed in contact with the surface of the tender leaves that have not yet expanded which undoubtedly

are more sensitive than the expanded leaves whose epidermis has already hardened."

According to Fawcett, then, the virus of sugar-cane mosaic is capable of passing through the epidermis of the leaves and does not require the breaking of the epidermis to reach the tissues inside. We know of no other experimenter who has obtained similar results.

Bonazzi (**5**), 1926, Director of the Chaparra Experiment Station of Cuba published the following method for transmitting sugar-cane mosaic which he used on seed pieces of one bud each:

A hole is bored diagonally into the node, immediately above the leaf scar and as close as possible to the bud, without thereby impairing this tissue. For this work a 3 mm. sterile cork-borer is used, the small core thus resulting being kept to seal the wound at the site of inoculation. In the hole thus prepared a small piece of fresh apical bud tissue, obtained from an infected cane, is introduced and crushed by means of a sterile glass rod. The hole is closed by means of the cork-borer core and the wound finally, hermetically sealed with a small cotton plug soaked in melted paraffin. Planting is done soon after inoculation. The infected tissue for inoculation should be removed from a freshly exposed apical bud only a few seconds before use, the cut being renewed for every prelevation of a new inoculum, in order to avoid undue aeration.

Dr. Bonazzi reports a high percentage of infection in the treated seed pieces and none in the checks but he does not state whether these results were constant upon repeating the experiment. Neither does he explain what is meant by "questionable infections" five of which he reports among his treated plants.

Working at the same time at the Insular Station in Porto Rico as Dr. Bonazzi in Cuba, but independently, Dr. M. T. Cook devised and tested a method for artificially transmitting sugar-cane mosaic which he has informed the author is capable of producing a high percentage of infection in susceptible varieties. The method is somewhat similar to that of Bonazzi. A one bud seed piece is cut out of a healthy stalk leaving the bud in the center and part of the internodes at each end. One end is then hollowed out by removing with a knife the spongy tissues down as far as the bud or close to it. About half of the seed piece is thus emptied out into a hollow cylinder. A plug is prepared from the uppermost joints of a mosaic cane stalk by removing the hard outer covering and forced into the healthy cylinder. The grafted seed piece is then planted.

McRae (**14**), 1927, and McRae and Subramanian (**15**), 1928,

in India have transmitted cane mosaic artificially by the following method: the cell contents of mosaic leaves were crushed out, then placed on leaves of healthy plants and pricked in with a sterile needle, controls having distilled water pricked in. In no case did the latter show any trace of disease, but mosaic was transferred from leaf to leaf or leaf sheath to leaf sheath in different canes; from various canes to maize and sorghum, but not to the lesser millets. This method seems to be the same used by Brandes, even though neither he nor McRae and Subramanian specify the kind of needle used. When inoculating from leaf of Co–213 to leaf of Co–213, McRae was able to obtain 8 infections out of 13; 8 out of 16 when inoculating from the same leaf to leaf sheath of the same variety of cane; and 16 out of 16 when inoculating from leaf of Red-Mauritius to leaf of Co–213. In no case did the controls produce mosaic markings. In contrasts to McRae's success it is interesting to remember that Brandes was able to obtain only 1 infection out of 20 inoculations using probably the same method.

No other method for artificially transmitting sugar-cane mosaic has been published up to date and the following statement by Hadden (**8**), 1928, sums up the situation: "Sugar-cane mosaic may be transmitted artificially by hypodermic injections, needle punctures, grafting, etc., but only with great difficulty and many failures."

Recently, at the Third Conference of the International Society of Sugar-Cane Technologists, held in Java, 1929, Dr. G. Wilbrink presented a paper to prove that sugar-cane mosaic can be transmitted by means of the knife used in cutting cane seed for planting. Dr. Wilbrinks's experimental plants, however, were exposed in the open to natural infection by *Aphis maidis* and the percentages of infection obtained were exceedingly low. It is claimed that the inoculum was carried and deposited on the cut surfaces of the healthy canes when they were sectioned later with the same knife. We have made a few tests of this method with complete failure and Matz (**13**) failed with a similar one. It seems rather improbable that it can produce infection.

This leaves as the most desirable and successful, the method used by Brandes and by McRae and Subramanian. This method, however, has not been tested under Porto Rican conditions.

A TEST OF THE METHOD USED BY BRANDES AND BY MCRAE AND SUBRAMANIAN

All the information given by Brandes (**1**) as to his technique is as follows: "Virus rubbed with fingers on unbroken surface

of young leaves. Youngest leaves inoculated by numerous needle pricks."

All that McRae and Subramanian (**15**) say is: "In the infection experiments given in Table VI juice was crushed from mottled leaves and immediately pricked into leaf-sheaths and stems of plants known to be free from mosaic disease."

Neither Brandes nor McRae and Subramanian specify what size of needle was used in making their inoculations. In testing their method under Porto Rican conditions we used a row of two-hundred cane plants of the variety SC–12(4) set in a plot at considerable distance from other mosaic cane plants. The plot was practically free from weeds, but of course, being in the open, a certain amount of error from infestation by *Aphis maidis* was to be expected. To obtain the virulent juice for inoculation, tender mosaic sugar-cane leaves of the same variety were cut up into bits with scissors, pounded in a porcelain mortar, the ground mass tied up in a piece of muslin and the juice extracted under a hand press. All the utensils were carefully washed and the juice was collected in a clean beaker and used immediately after extraction.

One hundred shoots each in a different plant were inoculated leaving every other plant in the row as checks. To perform the inoculation, the mature, expanded leaves were bent down and some stripped off exposing the tender parts of the central spindle of tightly rolled young leaves. A little of the virulent juice was then placed on the spindle with the finger tip and as the drop of liquid slid downward it was pricked in with a No. 0 Asta black insect pin. Three drops were placed on each spindle and numerous needle pricks made. The inoculations were made April 25, 1929. Up to May 18, 1929, 52 out of the 100 inoculated shoots had developed mosaic and by June 25, 1929, when the experiment was closed, the number of infections had increased to 56. As the mosaic pattern became clearly marked, the infected plants were pulled out of the row to prevent natural transmission. In spite of this precaution, however, one case of secondary infection appeared in the checks.

DISCUSSION

There can be no doubt that sugar-cane mosaic can be transmitted by pricking the virulent juices into the young leaves. It is nevertheless very likely that were we to repeat the experiment, the results would vary. The extraction of the virulent juices is a troublesome procedure and since the virus seems to be readily

destroyed or rendered non-infectious by exposure to the air the results would depend on the rapidity and care with which the work was done.

For the purposes we had in mind, the percentage of infection obtained by pricking in the virulent juices was not sufficiently high. The method itself was too troublesome for routine field work.

The direct pricking in of the inoculum from mosaic to healthy tissues had been attemptted by Earle (**6**) and by Bruner (**4**) with failure or low percentages of infection, but the instrument used by these investigators seemed to us to be too coarse. Disregarding the extraction of the juices as troublesome and subject to too much variation, we sought to devise a new method by improving on the work of Earle and Bruner through the use of a fine insect pin. In the new method as originally conceived, we depended on the spindle of tightly rolled tender mosaic leaves as the traditional source of the inoculum. Later, however, we found that the expanded leaves were a much more convenient and equally effective source of inoculum.

Evolution of the New Method

THE SPINDLE-TO-SPINDLE METHOD

Adhering to the traditional source of inoculum—the spindle or cylinder of tightly rolled tender leaves—our first attempts at devising a new method of artificial transmission consisted in holding a spindle pulled out of a mosaic cane plant tight against the exposed spindle of a healthy cane plant and running an insect pin through the mosaic into the healthy tissues. A No. 2 white Asta insect pin was used thrusting it rapidly in and out at several points on both spindles. The results were proportional to the care taken in performing the inoculations, the condition of the cane plants and possibly other factors. When vigorously growing cane plants were inoculated carefully and rapidly, this spindle-to-spindle method could be depended upon to produce about 70 per cent infection. The method, however, had several disadvantages. Only one spindle can be obtained from one mosaic shoot and it is troublesome to hold the spindles tightly together when performing the inoculation. We used it nevertheless, with satisfactory results during the years 1925–1928 on different sugar-cane varieties. During the year 1928, the method was simplified by the use of expanded mosaic cane leaves instead of the spindles of tightly rolled tender leaves as the source of the inoculum.

THE LEAF-SLIP METHOD

Contrary to the traditional belief, the expanded leaves of mosaic sugar-cane plants are as good a source of the inoculum as the more tender ones. Any leaf as soon as it has expanded can be used. The very young ones split easily and the older ones are not pliable. The medium leaves are therefore more satisfactory.

THE TOOL

Black number 0 and white No. 2 Asta insect pins have been used. It is possible that a finer pin than the black No. 0 but sufficiently stiff to enter the tissues without bending will produce higher percentages of infection and require a smaller number of pin pricks to be made at each inoculation, but for ordinary routine work those that have been used are very satisfactory. Ordinary pins have proved worthless since apparently they are too thick and short and allow the entrance of air into the wound.

TECHNIQUE

We ordinarily use one pin at a time to make the inoculations, but several can be tied together into a brush thus making many pricks at one time. When the plants to be inoculated are large and their spindles thick, the brush of pins is very convenient. Before using the pin we usually cleanse it by running it through cloth or the midrib of a cane leaf. No attempt has been made to sterilize the pin. No other disease than mosaic has developed in the inoculated plants as a result of the pin pricks and it is perhaps needless to add that mosaic does not result from pin pricks or from pricking in the juice of healthy cane leaves into healthy cane plants.

When sugar-cane plants from two feet in height and upwards are to be inoculated the procedure is as follows: The expanded leaves are bent down and the central spindle exposed. The outermost one of the leaves, the basal part of which is still tightly wrapped about the spindle, is stripped off exposing the whitish cylinder of tender leaves.

A slip about one inch wide and some eight inches long is stripped off a mosaic leaf, this is placed like a band around the base of the exposed spindle and held tightly with the thumb and forefinger of the left hand. The pin, held in the right, is thrust rapidly in and out repeatedly through different parts of the mosaic band into the healthy spindle. The pin is thrust in a slanting

position and is not made to go clear through the spindle. After the pin has been thrust in about twenty times, the mosaic leaf-band is moved upwards, held tightly again, and the pricking repeated at four or five points along the spindle using a fresh part of the mosaic leaf-band every time.

To inoculate very small cane plants whose stems are soft, the band of mosaic leaf is placed around the stem instead of the spindle. Corn and other grasses are inoculated in the same manner.

PERIOD OF INCUBATION

The period of incubation for sugar-cane mosaic is considered to be about fifteen days long. There is no difference in the length of the period between sugar-cane plants inoculated artificially by mechanical methods or through the agency of the insect vector, *Aphis maidis,* in nature.

The length of the period of incubation is measured by the appearance of the first symptoms of secondary infection. Since these symptoms appear in the tender leaves and not in those that were old or mature at the time of inoculation, it is obvious that the appearance of the symptoms depends on the rate at which the new leaves are growing and expanding.

ACCOUNT OF EXPERIMENTAL WORK

Only one experiment will be reported since it is representative of many others that were conducted. For lack of green-house facilities, they were all carried out in the open and therefore subject to error by the almost unavoidable liability to infestation by *Aphis maidis.*

A field near the Entomological Laboratory at the Insular Station, Río Piedras, P. R., was prepared in the usual manner and SC–12(4) cane planted in 14 parallel rows of 200 seed pieces each. The seed pieces were cut 1 foot long, planted 1 foot apart and the rows were spaced 3 feet apart. The seed came from one of the Station fields free from mosaic. After germination all the plants were examined and found to be healthy.

On August 24, 1928, the plants were about 4 feet high. All were inspected again and none were found with mosaic. In row No. 7, in the center of the field, 100 shoots, each in a different plant, were inoculated by the leaf-slip method and tagged. Alternate plants in the row were selected for inoculating thus leaving a check

in between. As each seed piece had produced an average of five shoots there were some 13,000 shoots in the plot.

From September 8 to September 15, 1928, ninety-four of the one hundred inoculated cane shoots in row No. 7 developed mosaic. As soon as the symptoms of secondary infection were evident in the inoculated shoots, the entire plant to which it belonged was pulled out of the field. Up to September 15, 1928, when the experiment was closed no other case of secondary infection had appeared in the field except the 94 that had been inoculated.

On September 20, 1928, while looking over the field, two cases of secondary infection were found in cane plants that had not been inoculated artificially. One of the cases was near the outer borders of the plot, one out of a stool of four shoots; the other was in row No. 7, also one in a stool of five shoots.

Up to the time the experiment was closed no weeds had been allowed among the cane in the experimental plot. The finding of these two cases of secondary infection, evidently the result of natural transmission through the agency of *Aphis maidis*, is not surprising since the plot where the experiment was conducted was surrounded on two sides by fields of "malojillo" or Para grass, *Eriochloa subglabra*, a host plant of the insect, and on the other two sides by fields in which other host plants were also growing. A low percentage of natural transmission is to be expected in any such experiment conducted in the open.

DISCUSSION

Brandes (**3**) reached the following conclusion on the requirements for successful inoculation from his studies on the transmission of sugar-cane mosaic by *Aphis maidis:*

> "It has been found that a definite, measurable quantity of 'Virus' is necessary. This would eliminate from consideration as an explanation the carrying into the plant of the scanty amount of virulent material adhering to the minute mouth parts."

Brandes in the same publication shows by means of sections how the setae of *A. maidis* are inserted into the tissues of the corn leaf:

> "Typically, the setae of *A. maidis* pass through the sub-stomatal cavity, then through the mesophyll cells, either intercellularly or intracellularly, continuing between two cells of the starch sheath and finally into the phloem of the vascular bundle."

In devising our new method for accomplishing artificially what

A. maidis does in nature, we have started from a point opposite to that of Brandes in that we have assumed that possibly the scanty amount of "virus" adhering to the mouth parts might be sufficient to produce infection if it were conducted to the healthy tissues without undue exposure to the air. Whether the "virus" has to be carried to the phloem to produce infection or not, this is accomplished mechanically by thrusting the pin into the leaves a large number of times.

For lack of greenhouse facilities we have not attempted to determine the number of pin pricks necessary to produce infection. This would require a very large number of inoculations under conditions in which there would be no possibility for *A. maidis* gaining access to the plants. We have made some experiments in the open field in which lots of twenty cane plants each were pricked once, twice and three times, at the base, center and upper part of the spindles through mosaic cane slips thrusting the pin in and out as rapidly as possible. All such inoculations gave negative results and were not repeated since the very low percentage of infection to be expected would be questionable as there is always in the open field the possibility of infestation by *A. maidis*.

When a hundred or more pin pricks are made into each plant, the percentage of infection is so high and so constant that the danger of infection through *A. maidis* can be overlooked. In the first experiment reported above we selected the row of plants to be inoculated in the center of the field to minimize as far as possible the danger of infestation by *A. maidis* crawling in from outside or dropping down from the air. Lacking green-house facilities, this was the best that could be done. To further correct possible error, the only means we had was the repetition of the experiment a large number of times. The percentages of infection in all these repeated experiments conducted during the years 1925–1929 was as a rule over seventy with contrasting exceedingly low percentages of infection in the checks.

With green-house facilities soon to be available at the Insular Experiment Station, the experiment will be repeated under perfectly controlled conditions.

The plants were considered mosaic as soon as the symptoms of secondary infection became well marked. The symptoms as described by Stevenson (**18**), Brandes (**2**), Lyon (**12**) and others are unmistakable. The incubation period of about fifteen days is the normal for the disease. During the period of incubation, the soil was moist and the plants in the experiment were growing vigorously.

In using a pin for performing the inoculation, the limiting factor seems to be the exposure to the air of the "virus" that is carried on the pin. Success therefore depends on how rapidly the pin is thrust in and out. When the cane plants are growing vigorously the tissues are turgid and the sap is flowing freely. This probably favors infection because the wound made by the pin seems to close up on it thus excluding the air and possibly because the "virus" is rapidly distributed through the plant. Thrusting the pin in a slanting position would also favor the exclusion of air from the wound.

When resistant or immune varieties of sugar cane are inoculated, other factors are concerned of which as yet we do not know much about. Some of the sugar-cane plants inoculated artificially by us in our experiments and some of the checks that developed mosaic through the agency of *Aphis maidis* were transplanted and kept under observation for several years.

There is no difference between sugar-cane plants inoculated artificially with sugar-cane mosaic and those inoculated in nature by the insect vector of the disease, the corn aphid, *Aphis maidis Fitch*. The period of inoculation is the same, (varying naturally with the rate of growth of the plants), the symptoms and the manner in which they appear are identical and the course of the disease is the same in either case.

SUMMARY

1. A new mechanical method for artificially transmitting sugar cane mosaic has been developed and used at the Insular Experiment Station of Porto Rico during the years 1925–1929.

2. The method is very simple and easy to operate and produces constant high percentage of infection.

3. Former investigators had sought to transmit sugar-cane mosaic artificially by extracting the virulent juices and injecting them into the healthy plant by means of a hypodermic needle syringe or by pricking them in after smearing the leaves. The extraction and injection of the virulent juices is always troblesome and the "virus" is quickly rendered non-infectious possibly by exposure to the air. Such methods besides being laborious and troublesome do not produce constantly high percentages of infection.

4. Bruner obtained a few infections by pricking in the virulent juices directly from the mosaic into the healthy tissues without previous extraction. The percentages of infection obtained were quite low. This was possibly due to the use of too coarse a needle.

5. The spindle of tightly rolled tender leaves has been the traditional source of the inoculum in artificial transmission experiments. Our new method (spindle-to-spindle) originally consisted in pricking in the inoculum from a mosaic into a healthy spindle held tightly together. This arrangement, though producing a constant high percentage of infection was somewhat cumbersome.

6. In its final form the leaf-slip method consists in pricking in the inoculum from a slip of mosaic cane leaf held tightly as a band around the exposed spindle of a healthy cane plant. To inoculate small cane plants, corn and other grasses, the band is held around the stem.

7. Black No. 0 and white No. 2 Asta insect pins have been used with equally good results. Ordinary pins do not produce the desired results.

8. The number of pin pricks necessary to produce infection has not been determined. Experiments in which groups of cane plants were pricked one, two and three times each, failed to develop mosaic. Ordinarily, about a hundred pin pricks are sufficient to insure infection.

9. The limiting factor seems to be exposure of the "virus" to the air. By thrusting the pin in and out rapidly in a slanting position a large number of times if there are any other requirements for infection, they will be fulfilled mechanically. The larger the number of pricks made, the larger the number of chances for infection.

10. Using a bunch of pins tied up into a brush makes the inoculation work more rapid, especially when inoculating plants with a thick spindle. Equally good results have been obtained with a bunch as with only one pin at a time.

11. Success depends on the care taken to shorten the exposure to the air, of the "virus" that is carried on the pin and on the rate at which the inoculated plant is growing. In resistant or immune cane varieties, other factors are involved, of which we as yet do not know much about.

12. When the inoculated cane plant is growing vigorously, the tissues are turgid and the sap is flowing freely. This seems to favor infection because (1) the wound made by the pin closes up on it thus excluding the air and (2) possibly because the "virus" is carried and distributed rapidly through the plant.

13. Inoculated cane plants of susceptible varieties, when growing rapidly show the first symptoms of secondary infection usually fifteen days after the inoculation. The symptoms appear and are

in every respect identical with those shown by plants that have been inoculated through the agency of the insect vector of the disease, the corn aphid, *Aphis maidis* Fitch. The course of the disease is the same in sugar-cane plants inoculated artificially as it is in plants inoculated in nature through the agency of the insect vector.

LITERATURE CITED

1. **Brandes, E. W.** The Mosaic Disease of Sugar Cane and Other Grasses. U. S. Dept. Agr. Bul. **829.** (1919.)
2. _____ Artificial and Insect Transmission of Sugar Cane Mosaic. Jour. Agr. Research **19**: 131–138. 1920.
3. _____ Mechanics of Inoculation with Sugar-Cane Mosaic by Insect Vectors. Jour. Agr. Research **23**: 279–285. (1923.)
4. **Bruner, S. C.** Sobre la Transmisión de la Enfermedad del "Mosaico" o "Rayas Amarillas" en la Caña de Azúcar. Rev. Agr. Com. & Tra. Cuba **5**: 11–22. (1922.)
5. **Bonazzi, Augusto.** Study on Sugar Cane Mosaic. Science, pp. 529–530. (Nov. 26, 1926).
6. **Earle, F. S.** The Year's Experience with Sugar-Cane Mosaic or Yellow-Stripe Disease. Jour. Dept Agric. Porto Rico **3**: 3–33. (1919.)
7. **Fawcett, G. L.** Apuntes sobre el Mosaico de la Caña de Azúcar. Rev. Ind. Agric. Tucumán **18**: 205–209. (1928.)
8. **Hadden, F. C.** Sugar-Cane Mosaic and Insects. Hawaiian Planters' Record **32**, No. 1. (Reprinted In Facts About Sugar, August 11, 1928, pp. 758–762.) (1928.)
9. **Kamerling, Z.** De Gele-Strepenziekte der Bladeren. Verslag 1902 Proefs. Suik. Oost Java "Kakok" to Pekalongan, pp. 76–81. (1903.)
10. **Kobus, J. D.** Vergelijkende Proeven Omtrent Gele-Strepenziekte. Arch. Java-Suikerindus., jaarg. **16,** pp. 350–374, (Reprinted as Meded. Proefs. Javasuikerindus., No. 12, 319–342). (1908.)
11. **Kunkel, L. O.** Studies on the Mosaic of Sugar Cane. Bul. Exp. Sta. H. S. P. A. Bot. Ser. **3**: 115–167. (1924.)
12. **Lyon, H. L.** Three Major Cane Diseases: Mosaic, Sereh, and Fiji Diseases. Bul. Exp. Sta. H. S. P. A. Bot. Ser. **3**: 1–43. (1921.)
13. **Matz, J.** Infection and Nature of the Yellow-Stripe Disease of Cane (Mosaic, Mottling, etc.). Jour. Dept. Agric. Porto Rico **3**: 65–82. (1919.)
14. **McRae, W.** Mosaic Disease of Sugar Cane in India. Agric. Jour. India **21**: 198–202. (1926.)
15. **McRae, W.** and **L. S. Subramanian.** A Further Note on the Mosaic Disease of Sugar Cane. Jour. Agric. India **23**: 239–255. (1928.)
16. **Menéndez Ramos, R.** Estudios sobre el mosaico de la caña. Revista de Agricultura de Puerto Rico. **13**: 219–220. (1924.)

17. **Smyth, E. G.** Annual Report, Division of Entomology. Ann. Rpt. Insular Expt. Sta. Porto Rico, **1919–20,** pp. 83–89. (1920.)

18. **Stevenson, John A.** The Mottling or Yellow-Stripe Disease of Sugar-Cane. Jour. Dept. Agric. Porto Rico **4** (i. e. **3**) : 3–76. (1919.)

19. **Stok, J. E. van der.** Verschijnselen van Tusschenrasvariabiliteit bij het Suikerriet. (Proeve eener verklaring der Gele-Strepenziekte en der Serehziekte.) Arch. Java Suikerindus., jaarg. **15,** pp. 581–601. (Reprinted as Meded. Proefs. Oost-Java S. 4, No. 36, pp. 457–477.) (1907.)

20. **Tower, W. V.** Mottling Disease of Sugar Cane. Rpt. Porto Rico Agr. Exp. Sta. **1919**, pp. 21–25. (1919.)

21. **Wilbrink G., and Ledeboer, F.** Bijdrage tot de Kennis der Gele-Strepenziekte. Arch. Suikerindus. Nederl. Indie, jaarg. **18**, pp. 465–518. (Reprinted as Meded. Proefs. Java-Suikerindus., deel 2. No. 39, pp. 443–495.) **(1910.)**

THE EFFECT OF SOME MOSAIC DISEASES ON CELL STRUCTURE AND ON THE CHLOROPLASTS

MELVILLE T. COOK

The early studies on the mosaic diseases of plants brought out two points which have been accepted very generally by most of the workers who have studied the histology and cytology of these diseases. They are: (1) the modifications of the cell structure of the leaf and (2) the modifications of the chloroplasts. Attention was called to the modification of the cell structure by Beijerinck in 1899, by Woods 1900 and 1902, by Iwanowski in 1903 and by many others since that time. In 1899 Beijerinck expressed the opinion that mosaic of tobacco was a disease of the chlorophyll bodies and finally became a disease of the plastic contents of the cells. In 1899–1900 König stated that the chloroplasts were disorganized and the cell walls disappearing. Unfortunately this idea was quickly followed by the very widespread belief that the chlorosis or mosaic diseases were due to the disintegration of the chloroplasts and this opinion is very generally reflected in the literature of the past thirty years. However, the fact that the chlorotic areas of many diseased plants gradually become green with age and exposure to sunlight tends to throw doubt on the theory of disintegration of the chloroplasts. Furthermore, it is very evident that this theory could not be applied to all types of virus disease of plants.

The author of this paper has made a study of these two phases of the subject and is of the opinion that the active agent in the mosaic diseases recorded in this paper is inhibitory in both cases. In the first case the cells of the chlorotic areas are inhibited so that there is a reduced growth with little or no differentiation of cell and tissue structures. In the second case the chloroplasts of the chlorotic areas are small and few in number as a result of the inhibitory action of the disease, but these areas tend to develop the normal green color with age.

This paper will be devoted to a review of the literature and to the results of the author's studies on these two points. However, it should be remembered that these studies do not include a study of the lesions which frequently occur in connection with virus diseases of plants.

CELL STRUCTURE

Mayer (**27**) published the first important paper on a mosaic disease of a plant in 1886 but he did not record any histological studies. Iwanowski (**19**) says that Mayer made microscopic examinations of the juices only.

In 1899 Beijerinck (**3**) published the results of his studies in which he made two important statements: (1) that the green areas were diseased and the chlorotic areas normal, and (2) that the dividing or meristematic cells only could contract the disease. The first statement is incorrect because we now know that the active agent exists in both green and chlorotic areas and because later studies show that the chlorosis is the most conspicuous symptom of the disease. The second statement is more nearly correct because recent researches have shown that the histological modifications occur only when the active agent comes in contact with the meristematic cells before the tissues are fully differentiated.

In 1899 and 1900 Köning (**23**) and (**24**) published the results of histological studies on mosaic tobacco leaves including drawings of cross sections but did not report anything of importance. His statement that intercellular cavities occurred among both the palisade and mesophyil cells leads one to suspect that his studies did not include the very young leaves.

In the year 1900, Heintzel (**17**) published a paper on tobacco mosaic in which he called attention to the greater prominence of intercellular spaces in the dark green, bloated areas than in the light green areas and expressed the opinion that these spaces were filled with gas. Later studies have shown that the intercellular spaces or the dark green areas are normal, or nearly normal, and that the reduced intercellular spaces in chlorotic areas are abnormal. Therefore, we are inclined to believe that Heintzel was influenced by Beijerinck's idea which was prevalent for a time, that the dark green areas were abnormal and the cholorotic areas normal.

In this same year Woods (**35**) published a paper on tobacco mosaic which gives the first clear statement that has come to our attention concerning the histological character of the chlorotic areas as compared with the tissues from normally green plants. He says:

"A study of the histology of the diseased leaves has now revealed a histological difference which makes it very clear that the light-colored areas are not normal, and this difference consists in the fact that in badly diseased plants the palisade parenchyma of the light-colored areas is not developed at all. All the tissue between the upper and lower epidermis consists of a spongy or respiratory parenchyma rather more closely packed than

normal. In moderately diseased plants the palisade parenchyma of the light area is greatly modified. Normally the palisade parenchyma cells of a healthy plant are from four to six times as long as broad. In a moderately diseased plant, however, the cells are nearly as broad as they are long, or at most not more than twice as long as broad. As a rule, the modified cells of the leaf pass abruptly into the normal cells of the green area. In a badly diseased leaf simply looking across the surface with the naked eye shows depressions where the light areas occur, or where the leaf is mostly diseased the dark green patches are raised above the general surface."

In 1902 Woods (**36**) also wrote as follows:

"Somewhat later I pointed out an important structural difference between the cells of the green areas and those of the light. In the latter, in the less pronounced cases of the disease, there is a shortening and broadening of the palisade parenchyma cells, and in the more pronounced cases there is an entire suppression of these cells, so that on simply looking across the surface of the leaf depressions are seen where the light areas occur and apparent blister-like development in the green areas. As a rule the modified cells pass abruptly into the normal cells of the green areas. The palisade cells of healthy leaves and of the healthy areas of diseased leaves are from three to four times as long as broad. Some plants are dark green, and these have the longest and narrowest palisade cells, closely packed with dark green chloroplasts, which seldom contain large starch grains. Other plants are much lighter in color. These have relatively broader palisade cells, approaching more in size and shape the spongy parenchyma cells of the same leaf. In both the light and the dark leaves the spongy parenchyma cells are about isodiagrametric, the diameter being about twice the length of the continuous palisade cells of the mature leaf."

In this same paper he commented on the differences in the cell structures of the chlorotic and green areas as follows:

"The conditions which cause the disease are certainly only effective when acting on meristematic or dividing cells. It cannot be induced artificially, and never appears naturally in cells which have stopped dividing. A diseased spot, therefore, never increases in size in a leaf except as the disease cells themselves enlarge. The mosaic nature of the trouble and the fact that under some conditions the plants may grow out of the disease suggests that it must arise in such cases in the meristematic tissues behind the growing point, or where the plant is not mosaic, but evenly diseased, the pathological condition must be present throughout the cells of the growing point."

In 1903 Iwanowski (**19**) published the results of extensive studies on tobacco mosaic and called attention to the error of Beijerinck who believed that the green areas was a true symptom of the disease. He says:

"When a diseased leaf is examined with care and compared with a sound one, a retardation of the development of the yellow spots is noticed; these are thinner than the greenish ones, and show a weak development on the obverse side."

He also says:

"A cross section of a green part gives the impression of a normal sound leaf, sometimes better developed than a sound one. The palisade and spongy parenchyma tissue are conspicuously differentiated.

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"We obtain a different appearance when the preparation is made from the yellow part of the leaf. In this place the leaf is thinner than the green one, in a proportion of 2: 3. It was believed that the palisade tissue was not yet developed, in its place we find other cells; a comparison with the green parts showing that the longitudinal walls have not been formed but are already in their development stage. The inter-cellular spaces are completely formed; so that we can differentiate at once the green from the yellow parts with which the weak tissue has correlation."

In this same paper he said:

"The parenchyma of the diseased leaves is more vigorously developed than those of the sound organs. In general the green parts seem hypertrophied and remind one of the forced development of tissue, on account of the presence of a parasite or insect injuries. This circumstance makes investigators of the mosaic diseases (Beijerinck) believe that the green spots are diseased and that the yellow are completely normal, and that they are only checked in their development or account of the deviation of the nutritive substances to the green parts of the leaves."

In this same year Bouygues (**4**) published a paper in which he reported the loss of the epidermis which was due probably to faulty technique.

In 1910 Westerdijk (**33**) published the results of studies on tomato mosaic, but the histological part did not add anything to our knowledge of the subject.

The next paper on this subject was by Melchers (**28**) in 1913 who made some studies on both tomato and potato. He reports that he did not find any abnormalities in the tomato, such as were described by Woods and Iwanowski for tobacco. However, he says—

"* * * at times in the yellow areas this tissue (palisade) appeared slightly less developed than usual. The yellow areas were slightly thinner than the adjacent green areas, especially in older leaves."

Concerning the potato he says—

"A microscopic study showed that the yellow areas were thinner at all ages; in some cases they were only 90 mic. thick as compared with 120 mic. in the normal leaf. This thinness was largely due to a shortening of the palisade cells which were of a striking cuboidal form. Sections from mottled areas were easily distinguished by the shape and size of the palisade cells. The cuboidal cells began very abruptly in some sections, while in other cases there was an intergradation between them and the normal palisade cells. In the yellow areas

as a rule, these cells were generally quite regular in shape, but sometimes there was less regularity. Their length varied from one-half to one-third that of normal cells and their thickness was usually slightly greater. The spongy parenchyma appeared normal in all areas, except that in the yellow regions, there were somewhat fewer chloroplasts."

In 1917 Chapman (**6**) published a bulletin in which he gives some attention to the histology of the tobacco leaves which showed mosaic. He agrees in general with Woods but says—

"Occasionally the dark green areas showed a more closely packed parenchyma than in normal leaves, and *always the palisade was well developed* and approached the normal in character. The development or non-development of the palisade layer, as Woods hinted, is dependent on the degree of severity of the disease. The lighter the attack the less are palisade cells and parenchyma tissue altered and *vice-versa*."

Chapman gives illustrations of cross sections from the normal tobacco leaf and from the green and chlorotic areas of mosaic leaves. These illustrations show that the palisade cells of the green areas and the intercellular spaces of the mesophyll are reduced as compared with the normal leaf. Also that the chlorotic areas are much thinner than the green areas, that the palisade is reduced to cuboidal cells and that the mesophyll is reduced and compact.

In 1920 Doolittle (**13**) published the results of his studies on the mosaic disease of cucurbits in which he described the anatomy of the leaves as follows:

"When stained sections of the leaves are examined, the palisade cells of the green areas are found to be crowded closely together and to be somewhat narrow and longer than those of the normal leaf. The palisade cells of the yellowed portion on the other hand, are more nearly isodiametric and less in number per unit area than in the normal leaf.

"The spongy parenchyma of these yellowed areas is also more compact and the intercellular spaces smaller than in the green portions."

In 1922 Matsumoto (**26**) published the results of his studies on the azuki-bean in which he said—

"The anatomy of the diseased leaves is found to be identical with that of cucumber mosaic as described by Doolittle. The mesophyll of the dark-green parts of the diseased leaves is strikingly thicker than that of the light green or yellowed parts. The palisade cells of the former are markedly longer and narrower than those of the normal tissues. The corresponding cells of the light-green parts are much shorter than those of the normal ones, more or less isodiametric, and show a tendency to crowd together so closely that their intercellular spaces are almost invisible. The second important difference between these two different portions occurs in the spongy parenchyma. The spongy parenchyma of the light-green parts is also strikingly compact as compared with that of the dark-green areas. The chloroplasts of the former, as in cucumber mosaic, are slightly smaller and less in number than in the darker areas."

In this same year Rand (**29**) published an extensive paper on the pecan rosette in which he says—

"Within the yellow thin areas between the larger side veins (secondary stage) the tissue is usually less developed than normal, and the cells are more closely packed together. In less severe cases the palisade tissue is well developed, but the intercellular spaces become almost obliterated. In other instances the palisade cells are differentiated, but the individual cells are greatly shortened vertically and may be only two or three times as long as broad, instead of seven to ten times, as in the normal leaf. In the most severely affected leaves with extreme variation in leaf diameter there is no differentiation into palisade and sponge tissue at all in the center of these yellow spots, but the tissue within these parts of the leaf consists entirely of closely packed, more or less isodiametric cells without conspicuous air spaces. Under these conditions the number of cell divisions may be somewhat increased, the cells remaining smaller than normal. Usually, however, the number of divisions is reduced, and in some instances a parenchyma tissue only three cells deep has been found. Occasionally the entire tissue between the margin and midrib consists of undifferentiated cells. That this lack of differentiation is not due to immaturity was shown by examination of young healthy leaves just after their emergence from the bud. Even at this young and only partially expanded stage the palisade tissue of healthy leaves was found to be well differentiated.

"In the thickened portions along the veins, or sometimes throughout the laminae in the aborted non-mottled leaves of depauperate rosettes, the size of cells may become increased in all three dimensions without essential change in ratio between spongy and palisade tissue. In some cases a slight increase in number of cell divisions also takes place. The individual cells appear abnormally healthy, and increase in leaf thickness here is due largely to increase in size of the individual cells. Along with this increase in cell size there is a reduction in size of intercellular spaces within the spongy parenchyma, and in the severely attacked leaf an almost complete obliteration of air spaces results.

"Linear leaves may or may not show a differentiation into palisade and spongy tissue, but where the palisade cells are developed they are usually shortened vertically and the spongy cells of the parenchyma are more closely packed together than the cells in healthy leaves. The average thickness of these leaves tends to be less than normal and this reduction is due partly to decrease in the number of cell divisions and partly to the shortened vertical axis of the palisade cells. The elongated shape of the leaves, however, is not due to variations in cell shape but rather to a decrease in number of cell divisions in which the central spindles are perpendicular to the midrib. This would tend to keep the cells closer to the main water supply of the leaf."

Various other workers have called attention to the reduced thickness of the chlorotic areas and to modifications of the cell structures, but the most extensive work to date was published by Dickson (**12**) in 1922. His work was especially valuable because of the large number of species and varieties of plants which he used. He also appears to have been the first to report that in some cases two layers of palisade cells were developed, which he believed to be a hyper-

plasia caused by a late or slight stimulation of the meristematic cells. In speaking of the tobacco he says—

"In a diseased leaf, however, there is a striking difference in the lamina thickness of the light and dark areas, the relative thickness being 180–200 and 270–300 microne, respectively, or a ration of 2 to 3. This is referable mostly to the difference in development of the palisade tissue in the two cases as was shown first by Woods. In the light areas the palisade parenchyma cells do not lengthen or do so to a limited extent depending on the severity of the infection. In most cases when leaves are severely infected the cells are cuboidal or isodiametric actual measurements showing variation from 20 to 60 microns in length and 17 to 28 microns in width. The cells of the spongy parenchyma in a healthy leaf are approximately twice as long as the palisade cells are wide, but in the light areas of diseased leaves the palisade cells are approximately equal in diameter to the underlying cells. This is due to the fact that the mesophyll cells are slightly smaller than in a healthy leaf, and that the normal division of cells in the palisade region is probably inhibited to some extent while the cells are meristematic. These cells are therefore in a hypoplastic condition as far as growth is concerned and the same applies to the cell contents as will be seen later.

"By contrast the palisade cells of the dark areas are well developed and in all cases they are at least normal in size. The writer has found, however, a very common modification when the disease is severe. Instead of the palisade cells occupying a single layer there are two layers, the lower comprising cells somewhat shorter than those in the upper layer but being obviously palisade cells. In such cases the individual cells are also narrower so that equal lengths of cross section from moderately and severely diseased leaves gave palisade cells in the proportion of 6 to 10, respectively, counting the upper row only in the latter. This palisade tissue consequently occupies a relatively greater volume of the leaf and there is a considerable increase in the number of cells. Since they are well supplied with chloroplasts able to function we may assume that the photosynthetic efficiency of this tissue is increased. In view of the increase in volume it is obvious that the palisade tissue in severely diseased leaves is in a condition of hypertrophy and because of the increase in number of cells the hyperthrophy may be accounted for by assuming a condition of hyperplasia when the tissue was meristematic. Woods considered the palisade cells of the dark areas to be normal for he states that the 'modified cells pass abruptly into the normal cells of the green areas'. Chapman however, while using the term 'healthy cells' say that 'their structure may be somewhat modified by the increased functioning thrown on the healthy cells'. It is the writer's view that when infection is severe in any given meristematic cell that cell will be hypoplastic, but if it is infected later, or only slightly, then it will be stimulated either to hypertrophic growth or to hyperplasia. This would account for such instances above mentioned."

Space will not permit the quoting of Dickson's studies on other plants but in his summary he says:

"Previous studies by other authors have been concerned chiefly with tobacco and in one or two cases with tomato and potato. In these no agreement is reached except in the finding of hypoplastic palisade tissue in lighter green areas of tobacco and potato and of fewer and paler chloroplasts in those areas.

"As a result of my studies with tobacco, tomato, potato, black henbane, petunia, pepper, sweet pea, Canada field pea, kidney bean, Windsor bean, raspberry, red clover, crimson clover, alsike clover, white Dutch clover and black medick it is clear that certain microscopic features are common to all the mosaic diseases studied. It is not to be understood, however, that in any leaf section from a diseased plant all characteristics, equally well developed, are to be observed. Much depends upon the growth rate and environment of the host plant but if sections are made from rapidly grown leaves of well-infected plants most of the following findings can easily be verified.

"There is a difference in the lamina thickness of light and dark parts of the leaf varying in amount and in the majority of cases giving a relative value of 2 to 3, respectively, where the disease is ordinarily severe. If the infection be slight there is less reduction in thickness of the lighter green parts but if the attack be unusually severe there is more reduction in the lighter parts and an increase in thickness of such parts as are dark green. Usually the dark green is but little thicker than an ordinary healthy leaf. This reduction in leaf thickness is due to hypoplasia of both palisade and spongy mesophyll cells but more especially to the former. There are all gradations from slight hypoplasia to that in which the cells are isodiametric and can only be determined as palisade cells by their position. It would appear that the hypoplastic condition of such cells is indicated also by the fact that they are usually somewhat wider that normal palisade cells. In a healthy leaf of tobacco, for example, a spongy mesophyll cell subtends approximately two palisade cells. Allowing that there is hypoplasia of the spongy mesophyll cells their reduced size is not very marked and yet only one hypoplastic palisade cell is needed to equal one mesophyll cell. This will be referred to again, but the point of view with regard to these palisade cells is that not only are they unable to develop in length but they must have been inhibited from further division when meristematic. In leaves where the attack is mild the palisade cells are narrower and longer, in fact nearly normal.

"The spongy mesophyll cells are also hypoplastic to a greater or less extent and when the disease is more severe they are equal in length to the width of the palisade cells above them. There is thus a very noticeable regular arrangement of the cells in the lighter green leaf parts."

* * * * * * *

"This regularity in cell arrangement necessarily reduces the proportion of intercellular space in the leaf, and by contrast there is usually an increase over normal in the intercellular spaces of hypertrophied parts."

Rawlins and Johnson (**30**) in 1925 published a paper on the cytology of mosaic tobacco in which they say—

"As has been found by other workers, the palisade cells in the chlorotic tissues are shorter and more nearly square in section than those in the non-chlorotic tissues of a mottled mosaic leaf or than those in healthy leaves."

In speaking of a section made from a plant which did not shown any symptoms of disease and in which the palisade cells were well developed, they say:

"A leaf of this age will probably never become badly mottled. At least part of the chlorotic appearance in mottled leaves seems to be due to the failure of the palisade cells in the chlorotic part to elongate. It is a common field observation that, when old plants are inoculated with mosaic virus, the older leaves do not become mottled, only the young growing leaves developing this symptom."

In 1926 Goldstein (**16**) published the results of extensive studies on the cytology of healthy and diseased tobacco plants in which she says:

"I find that the presence of the virus in the early stages in the histogenic development of the leaf results in the more severe types of mosaic patterns. Sections through the diseased leaves of tobacco plants, at all stages of their development, show that histogenic development was arrested in every case by the entrance of the virus. The distribution of the virus in the tissue is indicated by the stage in histogenic development which the various cell regions show. The lighter green regions of the leaf patterns were invaded before the dark green or normal green areas, which have continued their histogenic development to a latter stage. Sections through mature leaves, infected during the final stage of leaf development, show that the pattern is due to the effect of the disease upon the cell contents. In certain areas distributed through the leaf blade, such infections of leaves in later stages of histogenic development result in the faintly mottled apperance.

* * * * * * *

"In my opinion the dark green areas represent regions which have escaped infection in the early stages of the entrance of the virus into the leaf primordium. They are able to go through their histogenic development for a time, but soon the toxins of the virus hinder the completion of histogenesis, as far as differentiation is concerned. The cells continue to enlarge, and can only do so by elongating, since they are bounded by cells which, owing to the presence of the virus, are not enlarging to any extent, and are rather rigid. When the cells of the dark regions divide, they are likewise under pressure from the surrounding light green regions which are enlarging more slowly, so that a buldging upward to form a blistered or puckered region (savoying) results.

* * * * * * *

"As indicated in this seriation of the mosaic pattern types, the anatomical structure of a diseased leaf is correlated with the stage of its development when the virus entered it. When cuboidal cells are found in the light green areas of a given leaf, infection must have occurred at a very young stage and these cells have simply remained undifferentiated. The double palisade layer of the dark green areas, when studied from the developmental and histogenic standpoint, can be shown to be characteristic of young healthy green leaves at a certain stage of their development; neither has the hyperplastic development of a second layer of cells taken place, nor has hyperplasia of the cells themselves occurred in the dark green areas; the corresponding palisade cells of a leaf of mature development upon a mature plant may be much larger both in width and length than I have ever found them to be in the dark green regions of diseased leaves."

She also says:

"I find that Iwanowski's and Dickson's statements as to the histology of the dark green areas are not incompatible. Both conditions can be found, depending on the age of the leaf at the time when infection took place. My sections through mature healthy leaves of mature plants show that in tobacco as in many other plants, a second layer of less elongated palisade cells may normally occur. The term hyperplasia as implying the development of a second layer of palisade cells should not be used in connection with the dark green areas of diseased tobacco leaves. These areas consist of seven layers of cells such as are characteristic of healthy mature leaves.

"The cells found in the sections of the light green areas do constitute a hypoplastic condition due to severe infection, and represent the original embryonic condition of the leaf at the time when infection took place. Cytological study of very young leaves leads unquestionably to the conclusion that the entrance of the virus interferes with the histogenic development of the leaf. Embryonic cell layers of leaf primordia, which have been given an opportunity to develop before the entrance of the virus, show a normal palisade development. Furthermore, I find that the dark green areas, contrary to the opinion of Dickson are in reality also hypoplastic, and show a cell-form condition characteristic of a young stage in development of a normal leaf. The cells in such infected leaves soon cease to differentiate, and the palisade cells have simply elongated, producing the double palisade layer whose cells never attain the size of the cells found in mature healthy leaves. In accord with Küster's (1904) view of hyploplasia, such an arrested development should be considered as representing a hyporplastic condition."

In this same year Smith (**31**) published the results of her studies on mosaic diseases and leaf variegations. She states that the leaves of both healthy and diseased plants were of approximately the same age and the drawings show that these leaves were well advanced. Concerning her studies on *Datura stramonium* she says:

"It was found in the histological studies that neither the green nor the chlorotic areas of the mosaic-infected plants are comparable to the normal, the green being thicker than the healthy, but showing normal tissue differentiation and distribution of plastids; whereas the chlorotic areas are approximately equal in thickness to those of the healthy, but have poorly differentiated palisade tissue and show a decrease in the number of plastids."

Concerning the *Phytolacca decandra* she says:

"Here, as in *Datura*, it can be seen that the mosaic disease modifies the entire leaf, the dark green area being thicker than normal and the chlorotic region being reduced, particularly in the palisade layer. In this case also the healthy leaf is thinner than the dark green mosaic area but thicker than the chlorotic region. The thinness of the chlorotic sections is due chiefly to a lack of differentiation of the palisade layer."

She also reports a loss of the palisade cells in the chlorotic areas of a hybrid *Aquilegia*, a decrease in the thickness of the mesophyll

and that "the healthy leaves are thinner than the dark green areas and thicker than the chlorotic regions".

In 1927 Sorokin (**32**) published a paper on tomato mosaic in which she agreed with the finding of Dickson. Speaking of a chlorotic region she says:

> "All cells are nearly isodiametric; there is no differentiation into palisade and spongy parenchyma; the intercellular space volume is very limited; the epidermal cells are hardly distinguishable from the parenchyma cells. The chlorotic regions in less severely diseased leaves are characterized by the fact that the first row of parenchyma cells are slightly longer than those of the other three or four rows." * * * "The spongy parenchyma and intercellular spaces are present and the epidermal cells are somewhat hypertrophied."

So far as the writer is able to determine from the descriptions and illustrations, none of the investigators with the exception of Goldstein have given careful attention to the ages of the leaves which they have studied. In many of these papers it is very evident that the studies were confined to the old leaves in which the tissues were differentiated so far as possible.

It is unnecessary to call the attention of the readers of this paper to the fact that a young leaf is composed of undifferentiated cells which undergo rapid differentiation with the growth of the leaf, resulting in the well known tissues of the leaf, long before the leaf is full sized. It is reasonable to suppose that the reaction of the plants would vary somewhat with the age and development of these meristematic cells and more or less well developed tissues at time of coming in contact with the active agent which causes the disease. In studies recorded in this paper and in others by the writer, a series of leaves from the youngest in which the disease could be detected to the oldest in which the pattern was sufficiently well defined to permit the selection of material, were studied. Comparative studies were made between the chlorotic and green areas of the same leaf and between chlorotic areas of diseased plants and leaves of the same age from healthy plants. It is the opinion of the writer that these studies have made it possible to follow some of the reactions of the plants to the diseases with a fair degree of accuracy.

In general it may be said that chlorotic areas are thinner than the normally green areas and that these areas vary in thickness, not only in different plants but in different leaves, and also in different areas of the same leaf. In general it may also be said that the histological variations are not so pronounced in the monocotyledonous as in the dicotyledonous plants.

The differences in the thickness of the leaves of diseased and

healthy plants can be detected in very early stages of their development by studying buds from corresponding parts of diseased and healthy plants. Variations will be found frequently in a single leaf from a mosaic plant. Furthermore the cells in these thin areas are usually smaller than in normal leaves of approximately the same ages. These points are shown in figures 11 *a* and 11 *b* in which it will be readily seen that the diseased leaf (fig. 11 *b*) is thinner and the cells smaller than in the healthy leaf (fig. 11 *a*). As the leaves grow older the leaves from the healthy plant will develop the normal structure (figs. 12 *a*, 13 *a* & 14 *a*). The leaves from the diseased plant increase in size but the cell structure of the chlorotic areas are never normal. Sometimes they retain the structural character of undifferentiated cells throughout the life of the plant (fig. 12 *b*) while in other cases they approach the normal (figs. 13 *b*, 14 *b*, & 14 *c*). Sometimes they are almost normal in structure but modifications of the chloroplasts indicates that they are diseased.

Studies on other tobacco plants gave about the same history except that in most cases the chlorotic areas were thinner than the normal green leaves of the same age and the palisade poorly developed. The studies in general lead the author to believe that the poor differentiation in the first two leaves studied (figs. 11 *b*, & 12 *b*) as compared to the next two leaves studied (figs. 13 *b* & 14 *b*) was due to the fact that the first two leaves were younger at time of attack than the last two leaves.

The tomato plants used in these studies were infected with the same virus as the tobacco plants. The infections were severe and the symptoms very pronounced on the upper leaves; but the symptoms gradually disappeared with the increased age of the leaves. Several plants were used for study but all the drawings were made from the green and chlorotic areas of a single plant. The studies began with the youngest leaves in which the patterns could be detected with certainty. By this time, the palisade cells had developed in the green areas (fig. 15 *a*) but were very difficult to detect in the chlorotic areas (fig. 15 *b*). The chlorotic area was slightly thinner than the green area (figs. 15 *a*, *b*).

In the second leaf the development of the green area may be considered normal and complete (fig. 16 *a*). Sections from two chlorotic areas from opposite sides of the midrib of the same leaf (figs. 16 *b*, *c*) showed a variation in thickness, which is very common in all species that the writer has studied. The cell structure in both sections was very uniform, the palisade undeveloped and the intercellular spaces small.

The green area from the third leaf (fig. 17 *a*) was somewhat under developed as compared with the preceding leaf (fig. 16 *a*). The section from the chlorotic area (fig. 17 *b*) was slightly thinner than the green one (fig. 17 *a*), the palisade under developed and the mesophyll quite uniform.

The green part of the fourth leaf was thinner than the green parts of the third and second leaves but the structure may be considered normal or nearly normal. Sections from two chlorotic areas of this same leaf showed variations in thickness and structure (figs. 18 *b*, *c*). In one (fig. 18 *b*) there was practically no differentiation into palisade and mesophyll, while in the other (fig. 18 *c*) the differentiation was practically the same as in the green part.

The fact that the green areas of the third and fourth leaves (figs. 17 *a* & 18 *a*) were rather thin and under developed may or may not have been due to the disease. The author has noted that there may be considerable variation in the thickness of leaves of apparently healthy plants, regardless of ages. The partial development of the palisade cells in the chlorotic areas of the third and fourth leaves (figs. 17 *b* & 18 *b*) as compared with the lack of development in the first and second leaves (figs. 15 *b* & 16 *b*) is apparently due to the age of the leaves at time of attack. However it will be noted that sections from two chlorotic areas (figs. 18 *b* & *c*) of the fourth leaf show great variations in the development of the palisade. It is reasonable to suppose that this variation in development is due to an unequal distribution of the virus in the leaf.

The diseased cowpea plants used in these studies were very severely infected. Two sections from young normal leaves (figs. 19 *a* & *b*) and one section from an old leaf (fig. 20 *a*) show the line of development. In the very young leaf (fig. 19 *a*) the cell structure is undifferentiated. The differentiation is shown in figures 19 *b* and 20 *a*. The chlorotic areas in leaves of about the same age from diseased plants show remarkable variations in both thickness and structure (figs. 19 *c*, *d*, & 20 *b*, *c*, *d*, *e*). Here as in the tomato we find that two chlorotic areas from the same leaf may show variations in both thickness and structure. This is illustrated in figures 19 *c* and *d* which are from the same young leaf. Figure 19 *c* shows what is to be expected in a severely infected leaf; there is practically no differentiation. Fig. 19 *d* shows some differentiation but the condition of the chloroplasts indicate the infection. The variations in an older leaf are shown in figures 20 *b*, *c* and *d*. The section shown in figure 20 *b* was from a slightly chlorotic area. The thickness is the same as in the normal leaf (figure 20 *b*), the palisade consists of two layers of cells and the mesophyll

cells are somewhat smaller and more compact than in a normal leaf or green area of a diseased leaf. Figures 20 *c*, *d*, *e*, were made from sections from old, severely diseased leaves. They are thinner than a normal leaf of the same age (fig. 20 *a*). The cells are rather large but meristematic in character except in figure 20 *d* in which there is a tendency towards the development of a palisade. These variations are probably due to the age of the leaves at the time they were penetrated by the active agent, or to the virulence of the active agent, or to unequal distribution of the active agent.

DISCUSSION

It is very generally recognized that the active agent is distributed throughout all parts of the plant, except the seeds, in which it has been found in only a few cases. It is also very generally recognized that the chlorotic areas are thinner than the green areas of the same leaf or a normal leaf of the same age. These studies show that these chlorotic areas may vary in thickness, even in the same leaf. In brief a leaf from a mosaic plant of tobacco, tomato or cowpea may show variations in thickness and structure ranging from a green area of normal thickness to a chlorotic area which is very thin and composed of undifferentiated mesophyll supported by an imperfect fibrovascular structure. All gradations in thickness and structure may occur between these two extremes. In some cases the only modification of structure is the formation of two layers of palisade cells.

So far as the author has been able to learn, Woods was the first to call attention to the reduced thickness of the chlorotic areas and this has been confirmed by most investigators since that time. He also called attention to "the fact that in badly diseased plants the palisade parenchyma of the light colored areas is not developed at all. All the tissue between the upper and lower epidermis consists of a spongy or respiratory parenchyma rather more closely packed than normal". He also stated that "the conditions which cause the disease are certainly only effective when acting on the meristematic or dividing cells".

Since that time many investigators have confirmed Wood's opinion that chlorotic areas were thinner than the green areas and that the palisade was undeveloped. However, no one appears to have been impressed with the idea that the structural variations were due to the action of the active agent on the meristematic tissue until the publication of Dickson's paper (**12**) in 1922. He said:

"It is the writer's view that when the infection is severe in any given

meristematic cell that cell will be hypoplastic; but if it is infected later, or only slightly, then it will be stimulated either to hypertrophic growth or to hyperplasia."

The writer agrees with the first statement of Dickson but not with the latter. The studies on which this paper is based indicate that the active agent which causes the disease is inhibitory. It produces a hypoplasia by inhibiting the differentiation of the meristematic tissues. The writer does not find hypertrophy in any of the cases studied, which are usually classified under the general term of mosaic.

So far as the writer has been able to learn Goldstein (16) was the first to assert that "the virus interfered with the histogenic development of the leaf" and that "the distribution of the virus was indicated by the stage in histogenic development". The studies of the writer very generally confirm the results reported by Goldstein.

It is very evident to the writer that the active agent inhibits the growth and differentiation of the meristematic cells. It is also evident to the writer that the active agent does not cause a change on any of the leaf tissues after they have completed their normal development. The variations in the histogenic structures found in the different chlorotic areas as recorded in this paper may be due to any one of several causes:

1. The amount of the virus or active agent injected into the plant and carried to the meristematic region.
2. The growth activity of the host plant at the time.
3. The relative virulence of the active agent.
4. The relative age of the tissue at time when it comes in contact with the active agent.
5. The unequal distribution of the active agent throughout the leaf.

The growth of the tissues of chlorotic and green areas is not the same. In some cases the differences in growth are so great as to cause a deformity. This is especially noticeable in the tobacco where it results in the formation of pockets in the green areas. This pocketing is evidently due to the fact that the growth in the chlorotic areas has been slowed up while that of the green areas appears to be normal or nearly normal.

The differentiation of the tissues in the chlorotic areas is inhibited and the growth and division of the cells is slowed up to some extent. The result is that the chlorotic areas increase in size as a result of the division and growth of the cells. However, there is no evidence to indicate that it increases in size as a result of the invasion of the

active agent after the tissues are mature. Woods (**36**) appears to have been the first to call attention to this point when he said:

> "A diseased spot, therefore, never increases in size in a leaf except as the diseased cells themselves enlarge."

The writer of this paper confirmed this idea for sugar cane in a paper published in 1926 (**10**) by the following statement:

> "These areas increases in size with the growth of the leaves, but so far as the writer has been able to determine they do not encroach on the surrounding tissues and do not coalesce. The increase in size appears to be by cell division and growth and not by infection of surrounding cells."

CHLOROPLASTS

It is very generally recognized that the very young leaves of most plants are white or very light green and that those which have a mosaic disease show very faint or no mosaic patterns whatever until they have been exposed to sunlight and begin to turn green. In the case of normal plants the young leaves become uniformally green very rapidly when exposed to sunlight. In the case of plants infected with mosaic, certain areas become normal green very quickly while certain others remain light green or yellowish for a time displaying the well known pattern of mosaic and then in most cases gradually become green until the pattern almost or completely disappears. This has been observed by a number of writers and appears to be true for most plants. The most notable exceptions are the lettuce as reported by Jagger and the *Abutilon hirtum* which will be discussed by the writer in a later paper. Jagger (**20**) in his discussion of the lettuce mosaic says:

> "The first symptom of disease was a yellowish discoloration along the smaller veins of the younger expanded leaves. This symptom was usually evident for only a few days, giving way to a general yellowish, discolored appearance of the whole plant. All gradations of discoloration occurred from very marked to conditions not distinguishable with certainty from normal."

This statement indicates that the discolorations become more pronounced with age which is not in harmony with most descriptions of mosaic symptoms; but he further says:

> "Often plants that showed marked discolorations, mottling, and stunting soon after becoming diseased would later seem to recover in part and to make a more or less normal growth with only slight discoloration and mottling."

This last statement indicates that the patterns disappear in some cases and the plants tend to become green with age as described for

most mosaic diseases. In the *Abutilon hirtum* which will be discussed in a later paper some of the spots become slightly greener with age while others remain chlorotic.

This tendency for the chlorotic areas to become green with age and exposure to sunlight led the writer to doubt the generally accepted idea that the chlorotic condition was the result of a disintegration of the chloroplasts as stated by several writers. If the chloroplasts undergo disintegration, these chlorotic areas ought to become more chlorotic instead of green. Therefore, the writer began a series of studies on this phase of the mosaic problem some years ago and the results were first presented in a paper published (**9**) in 1925. This was followed by a paper (**11**) which was read before the Philadelphia meeting of the American Phytopathological Society in 1926 and published in 1927 (**10**). These papers were received with more or less differences of opinion. The writer having made his studies almost entirely on sugar cane, which had not been used by any of the other workers on this phase of the subject, decided to continue the studies and to use a number of other species of plants. The plants used were the common red canna (*Canna indica*), tobacco and tomato, all of which have been studied by other workers, cowpea which has been studied to some extent and *Abutilon hirtum*. The readers will note that the sugar cane and canna are both monocotyledonous while all the other are dicotyledonous. This may be of some importance because in the first two the leaves are rolled while in all the others they are folded and because the cell structures are somewhat different.

Before giving the results of these studies on chloroplasts, it is desirable to give a brief review of the literature to date. Mayer (**27**) referred to the chloroplasts in mosaic of plants and said that mosaic was a disease of the plasmatic cell contents. Köning (**23** & **24**) reported a disorganization of chloroplasts and a disappearance of the cell walls, which leads the writer to suspect that he studied old leaves and that the conditions were due to other causes. The statement that the chloroplasts were disorganized or disintegrating appears to have been generally accepted until the writer of this paper published (**9**) evidence to the contrary in 1925 (see page 88).

Beijerink (**3**) defined the mosaic of tobacco as a disease of the chlorophyll bodies which finally became a disease of the plasmatic contents of the cells. However, he did not give an explanation of his results. The writer is of the opinion that he also was working with old leaves.

Woods (**35**) in the conclusions of his first paper makes the very

definite statement—"Chlorophyll is destroyed rapidly by oxidizing enzymes, of which two groups are recognized,—oxidases and perioxirases", but he does not explain why the chlorotic areas become green with age.

In 1902 he said (**36**):

"That the disease is not primarily of the chloroplasts, as Beijerinck thought, is evident from the fact that in the less pronounced cases the chloroplasts, though fewer in number are not decreased in size or activity. * * * In some pronounced cases the chloroplasts are light colored or wholly without color."

Iwanowski (**19**) published a paper in 1903 in which he said:

"If we now turn our attention to the cell contents, there is a lack of chloroplasts which are not regularly distributed in the palisade parenchyma. Investigation on the live-stage shows swollen chloroplasts which have the appearance of big drops with green, fine circles around them. Sometimes these drops fill the whole cell, rarely the chloroplasts are scattered and the cell contents is of a pale green color. As in the beginning of the observation and because of the rapid preparation of the solution, such formations appear rarely, we have to accept that they are more properly an artificial product, a continuation of the resistance capacity of the chloroplasts of the yellow parts in comparison with the green. In the last ones no such scattering of the chloroplasts has been seen.

"The chloroplasts on the yellow parts have a pale yellow color, with a granular structure. At first there is no vigor, cuts on new leaves treated with iodine showed a great difference; in the green parts an intense color, in the yellow, not a vigorous granule. Examinations with the naked eye showed that a diseased leaf after being treated with iodine showed color in dark blue and yellow sections, while the dark blue sections corresponding to the green parts. Later on, the vigor shows on the yellow parts also."

Baur (**1**) reported in 1904 that in *Abutilon thompsoni* there was no evidence of a causal organism but that the plastids in the chlorotic areas were reduced in size and number.

In 1910 Westerdijk (**33**) reported the chloroplasts of tomato mosaic as slightly smaller than normal, yellowish and containing but little starch. Melchers (**28**) reported in 1913 that the chloroplasts in the chlorotic areas of mosaic potatoes were fewer and of a pale yellowish green. Doolittle (**13**) reported in 1920 that the chloroplasts in the cells of the chlorotic areas of the cucumber were smaller than in green leaves and that they were located so very close to the cell walls as to be almost invisible.

In 1915 Clinton (**7**) said:

"Very frequently, especially where the chlorosis is of the mosaic or variegated type, it seems to start in the very young tissues, and not after the leaves are fully grown.

In 1920 Doolittle (**13**) published a paper on the mosaic of cucurbits in which he said:

"The chloroplasts of the cells of the yellowed portions are decidedly smaller than in normal cells and are often pressed so closely to the walls of the cells as to be almost invisible. In contrast to this the chloroplasts in the dark-green areas are so large and numerous as to seem to fill the cells. The vascular elements of mosaic show little variation from the normal."

Kunkel in 1921 in his paper (**25**) on a mosaic disease of corn says:

"Even before the leaves unfold and while they are still quite white the diseased areas appear translucent and can readily be distinguished from the portions of tissue, which on exposure to light will become deep green. As the leaf unfolds in the light and becomes green the outlines of the diseased spots or stripes become more clearly marked. The light color of the diseased areas is due to a failure of these portions to develop the normal green color rather than to a fading of the green color after it has once been produced."

Dickson (**12**) in his studies on a large number of plants frequently refers to the reduced number of chloroplasts in the chlorotic areas but shows that he is a believer in the disintegration theory by several statements. In his discussion of the tobacco mosaic he says:

"The carbohydrate content is not the same in light and dark-green areas is indicated by the iodine test. The chloroplasts and grains in the lighter-green areas take on a brown stain, even when there is little obvious change in the chlorophyll apparatus, and the amount of carbohydrate is less because of the fewer chloroplasts.

"The chlorotic condition of the lighter areas is not due to lack of iron in the plastid as is clearly indicated in the following tests."

After giving these tests he continues:

"The only cells in which this was difficult to demonstrate for individual plastids were those containing many tiny hyaline masses resulting from their break down. It is possible of course, that in such cases we have a hypoplastic condition under which the normal plastids did not develop originally but it appears more probable from my observations that this is a case of degeneration."

In his discussion of the potato mosaic he says:

"The chloroplasts of the cells of the light areas are fewer in number and paler in color than in the dark green but there are fewer instances of chloroplast degeneration than in tobacco. In the dark green tissues the chlorophyll content is always at least equal to the normal and sometimes above that of healthy tissue."

Again in his summary of the results of microscopic studies (p. 49) he says:

"'Corresponding with the hypoplastic condition of the cells there is a gradation of hyploplasia and degeneration of the cell contents. Cells, slightly

affected as far as growth is concerned may be different from healthy cells only in the slightly paler green of the chlorophyll while the chloroplasts appear to be present in nearly normal number. With increase in severity of effect on cell size there is a very definite reduction in the number and of depth of color of the chloroplasts and in some instances they are also reduced in size."

Johnston (**22**) says in speaking of sugar cane that—

"This mottling character is most clearly seen in the inner newly developed leaves, often fading out in the mature leaves. It is not seen in any very young shoots."

He also says—

"A gradual disappearing of the mosaic in old leaves."

In 1925 Rawlins and Johnson (**30**) published their studies on the cytology of mosaic tobacco in which they said:

"From the drawings it is evident that in this leaf the palisade cells and their chloroplasts are well developed. A leaf of this age will probably never become badly mottled. At least part of the chlorotic appearance in mottled leaves seems to be due to the failure of the palisade cells in the chlorotic part to elongate. It is a common field observation that, when old plants are inoculated with mosaic virus, the older leaves do not become mottled, only the young growing leaves developing this symptom."

In the writer's first paper on this subject in which the studies were restricted almost entirely to sugar cane attention was called to smaller number and small size of the chloroplasts in the chlorotic areas as compared with the green areas and normal cane. The writer said:

"A study of the cells of these leaves shows that the chloroplasts are more numerous in the dark geen than in the light green areas. Therefore, it appears that in the case of the light green areas the formation of the chloroplasts is inhibited but that with the exposure to sunlight this inhibition is gradually overcome.

* * * * * * *

"The chloroplasts in the white or yellow areas were fewer in number and smaller than in the green areas.

* * * * * * *

"The small chloroplasts of the mosaic cells are usually spoken of as having undergone a process of degeneration. The writer has studied the chloroplasts in the very young unrolled leaves and is satisfied that these chloroplasts are undeveloped rather than disintegrated. They have never reached normal development. Furthermore a study in a series of leaves from those just unrolling to the outermost on a plant shows that the chloroplasts of both normal and mosaic cells increase in size after being exposed to light. These changes which are apparently due to age or light are so pronounced that there is very little difference in size of chloroplasts in old leaves of diseased plant and the chloroplasts

in a leaf of the same age from a healthy plant. The writer is very doubtful if disintegration over occurs in case of primary infection (*i. e.* infection from the seed)."

In the paper (**11**) read before the Phliadelphia meeting (1926) of the American Phytopathological Society the writer expressed the opinion that—

"The chloroplasts are not destroyed nor even injured by the diseases. Their development however is inhibited."

In the more complete paper (**10**) on this subject the writer said:

"The chloroplasts in the sheathing cells of a healthy plant are large and the markings well defined as compared with the chloroplasts from the corresponding cell of a diseased plant which are small and with poorly defined markings. The chloroplasts in the ordinary mesophyll cells of a healthy plant are large and regular as compared with the chloroplasts from the corresponding cells of a diseased plant. However, as previously stated, the chloroplasts in the chlorotic areas in the older leaves of a diseased plant are very nearly or quite normal in appearance. No disintegration of the chloroplasts was observed in any case except in very old leaves which were decaying from old age or in tissues which were decaying as a result of attacks of fungi."

The writer also said:

"The tendency of leaves of diseased plants of both sugar cane and tobacco to become more or less uniformily green with age and exposure to sunlight is no doubt due to tendency of the chloroplasts in the cells of the chlorotic areas to increase in number and size. This could not possibly be the case if the chloroplasts were undergoing disintegration."

In 1926 Eckerson (**15**) reported the finding of flagellate organisms living in and destroying the chloroplasts in plants infected with tomato mosaic.

In this same year, Jones (**21**) described a mycetozoan (*Plasmodiophora tabaci*) from tobacco plants. He says:

"Disintegration of chloroplasts apparently is the first visible response on the part of the cell to the presence of the organism. Later the cell walls disintegrate and disappear."

In the following year Sorokin (**32**) published a paper on "Phenomena Associated with the Destruction of the Chloroplasts in Tomato Mosaic" in which she gives a short review of the literature and a lengthy discussion of her experiments.

In the summary of the paper Sorokin says:

"The chloroplasts are destroyed through the dissolution of the proteins of the stroma. This fact is easily demonstrated by direct observation of the living material and by microchemical tests. The same is confirmed by study of the

material fixed under the microscope, or fixed and stained by usual cytological methods.

"The normal chloroplasts are rather solid. The first indication of a pathological condition is the appeearance of rapidly moving hyaline bodies within the chloroplasts. The movement of these bodies is possible only after liquification of the stroma of the chloroplasts has taken place. Therefore it is assumed that a proteolytic enzyme, possibly secreted by an organism, is present. An increased osmotic concentration results inside of the chloroplasts, and water is taken in from the surrounding medium. Finally, the entire body of the chloroplasts goes into solution and, if sufficient water is present in the surrounding medium, spherical transparent vesicles result.

"The sphere represents a membrane resulting from the digested chloroplast in which the digestion products and water are retained, the whole being surrounded by a surface film at the interface. The spheres give negative protein reactions, and are soluble in alcohol, acetone, or acids. They are soluble in weak alkali. They can be fixed and stained by methods used in the study of the inclusion of the animal virus diseases."

In this same year Bremer (**5**) published the results of his studies on the cytology of the stripe disease of sugar cane and other plants in which he gave some attention to the chloroplasts. He reports a reduction in the size of the chloroplasts in some species and varieties but not in others. In one place he says:

"The chlorophyll grains are very irregularly scattered and are absorbed to a large extent by the stripe-diseased body"

which indicates a belief in the theory of disintegration.

In another place he says:

"I also found that, in most cane species, the chlorophyll had a lighter green color, in the light-green areas, the chlorophy grains not having reduced in size. In Betoeng I Rokan and Kapoer I Rokan, both species showing entirely white stripes on the leaves, most of the chlorophyll grains had remained small, owing to the disease. There even were chlorophyll grains that could hardly have been seen when strongly magnified, while they were entirely wanting in many cells. The very small chlorophyll grains were colorless. In mosaic Hippeastrum and Eucharis, I found also that in cases where the color of the leaves was lighter green, the chlorophyll grains had remained smaller than in healthy plants."

However, his figure No. 1 shows a healthy cell in which the chloroplasts are large and a diseased cell in which they are small.

In 1927 Hoggan published the results of her studies on the virus diseases of Solanaceous plants (**18**) in which she made the following statement:

"Where the external symptoms of mottling or chlorosis were marked, the chlorophyll had changed from green to yellow, and the plastids were reduced in size, in severe cases of disease sometimes disintegrating and apparently breaking up into small particles. In leaves infected when very young, the chloroplasts did not develop normally but remained small and fewer in number than in the

healthy cell. When placed in water, these plastids often swelled up to form colorless vesicles as described by Iwanowski (19), and it is thought possible that this may have some bearing on the formation of the 'spheres' reported by Sorokin (32)."

This brief review of the literature will show that the theory of disintegration of the chloroplasts as advanced by Köning and Beijerinck was very generally accepted by most workers without question until 1925 when the writer (9) advanced the theory of inhibition.

This theory of inhibition of the chloroplasts was well received by some workers but was not accepted by others. This difference of opinion led to the more extensive studies which are reported in this paper. However, before taking up the additional data we will review the results of the work on sugar cane as given in the authors last paper. In this connection the attention of the reader is called to the fact that the leaves of the sugar cane do not have a well defined layer of palisade cells such as is found in the leaves of most plants. However, the leaves of the sugar cane do have two distinct types of parenchyma cells: (1) a single layer of long tubular cells forming a sheath around the fibro-vascular bundles, and (2) ordinary parenchyma cells which are irregular in size and form and constitute the true mesophyll of the leaf.

Cross sections of leaves of healthy plants beginning with the inner leaves (figs. 1 *a*, 1 *c*, 1 *e*)* show that the number and size of the chloroplasts is practically the same throughout the series from oldest to youngest leaves. This is also shown in longitudinal sections through these sheathing cells (figs. 3 *a*, 3 *c*, 3 *e*) and in sections through the ordinary mesophyll cells (figs. 5 *a*, 5 *c*, 5*e*). But similar sections through the chlorotic areas of mosaic leaves of the same ages and positions as the leaves on the healthy plants showed that the chloroplasts were few and small in the cells of the inner leaves but increased in size and number in successive leaves from the youngest or innermost to the oldest or outermost leaf (figs. 2, 4 and 6). These studies were made on a number of different plants and the results were always the same. There was no evidence of disintegration of the chloroplasts except in the very old leaves in which some of the cells were abnormal as a result of old age or the attacks of fungi. All these studies were made from sections cut from leaves fresh from the plant and studied immediately and from sections made from leaves which had been killed and fixed a few minutes after collecting.

It appears to the writer that this tendency of the chloroplasts to

* This series includes five leaves. The drawings have been made from numbers 1, 3 and 5. However, studies have been made on a series of seventeen leaves from a single plant.

become normal in number and size is due to the exposure to sunlight rather than to age. Both normal and diseased canes were germinated and grown so far as possible in a dark room and both remained chlorotic.

The mosaic disease was discovered in our plots of red canna (*Canis indica*) but unfortunately all the plants were diseased and it was impossible to compare the normal with the mosaic plants. The patterns disappeared very quickly with age and exposure to sunlight, so that is was impossible to find the patterns on the fifth leaf. However, it was very easy to compare section from the green and light colored areas of the four youngest leaves. Sections were made from the leaves that were just unrolling as soon as the patterns were sufficiently distinct to show the boundaries and from the second, third and fourth leaves. No sections were made from the fifth leaves because the patterns were practically lost and because they were infected with rust fungi (*Puccinia cannae* (Went.) P. Henn.)

An examination of sections (fig. 7 *a*) from the green parts of the innermost or youngest leaves showed numerous, well developed chloroplasts while an examination of sections (fig. 10 *a*) from the green parts of the fourth leaf showed no differences or only slight increases in size and number of chloroplasts. An examination of sections (fig. 7 *b*) from the chlorotic areas of the inner or youngest leaf showed very few and small chloroplasts, while an examination of sections from the light areas from the second (fig. 8), third (fig. 9) and fourth (fig. 10 *b*) leaves showed a gradual increase in the number and size of the chloroplasts from the innermost or youngest to the oldest or outermost of the leaves. This is in harmony with the conditions recorded for the leaves of the sugar cane and indicates that there is a true inhibition of the development of the chloroplasts in the chlorotic areas which is partially overcome by age and exposure to sunlight.

Sections were made from a series of leaves of a healthy tobacco plant beginning with very small leaves and ending with leaves about seven inches in length (figs. 11 *a*, 12 *a*, 13 *a*, 14 *a*) showed a gradual increase in the number and size of the chloroplasts. This differs from the sugar cane and canna in the fact that the chloroplasts are fewer and smaller in the young than in the old leaves. A study of sections from the chlorotic areas of corresponding leaves from diseased plants showed that in the youngest leaves (fig. 11 *b*) there were no chloroplasts, in the second leaf there were a few small chloroplasts (fig. 12 *b*), while in the third leaves there was an increase in number and size of the chloroplasts (fig. 13 b) but that the increase was not so

great as in the corresponding leaves from healthy plants (figs. 12 *a*, 13 *a*). However, in the fourth leaves from the diseased plants (figs. 14 *b*, 14 *c*) the chloroplasts were almost equal in number and size to those in the healthy leaf (fig. 14 *a*). The presence of the palisade layer of cells the third and fourth leaves (figs. 13 *b*, 14 *b* and *c*). The short palisade cells in 14 *c* indicates a more severe infection than in 14 *b*. The tendency of the chloroplasts to increase in size and number with age and exposure to sunlight is in harmony with the preceding studies.

In the study of the tomato plants there was some doubts as to whether any of the plants were absolutely free from the disease. Therefore, it was considered advisable to compare sections from the green and chlorotic areas of the same plant. The youngest leaf in this study was not so young as the youngest leaf used in the tobacco studies as shown by the fact that the palisade in the green area of the youngest leaf (fig. 15 *a*) was farther advanced than the palisade in the green area of the youngest leaf of the tobacco (fig. 11 *a*). The chloroplasts in the green areas of the tomato plant were very numerous and fairly regular in size in all the leaves from the youngest to the oldest (figs. 15 *a*, 16 *a*, 17 *a*, 18 *a*). However, the cell structure of both the third and fourth leaves (figs. 17 *a* and 18 *a*) indicates a slight infection as compared with the second leaf (fig. 16 *a*). In the chlorotic area of the first leaf (fig. 15 *b*) there were no chloroplasts; in the chlorotic areas of the second leaf (fig. 16 *b* and *c*) the chloroplasts were few and small. In the section from a chlorotic area of the third leaf (fig. 17 *b*) which showed a palisade layer, indicating late or slight infection, the chloroplasts were larger and much more numerous than in the second leaf. Sections from two chlorotic areas of the fourth leaf (figs. 18 *b* and *c*) show an increase in number and size of chloroplasts. These sections also show a condition which is quite different in both the tobacco and the tomato in that the structural development is more advanced in one than in the other, which the writer interprets as indicating that the infection was either more severe or earlier in the one than in the other. It should also be noted that in the section from a chlorotic area in which the palisade was well developed (fig. 18 *c*) the chloroplasts were fully as abundant as in the sections from green areas (fig. 18 *a*) but that they were much smaller. In this as in the preceding, the study of the sections indicates an inhibition of the chloroplasts which is partially overcome with age and exposure to sunlight.

The studies on the cowpea were less extensive than on the other plants but included both healthy and diseased plants. The very

youngest normal leaf studied did not show any differentiation into the palisade and mesophyll yet the chloroplasts were numerous and large (fig. 19 *a*). In a slightly older normal leaf in which the palisade was fairly well developed the chloroplasts were equally numerous and larger (fig. 19 *b*). In a fully developed normal leaf the chloroplasts were abundant and considerably larger (fig. 20 *a*). Two sections (figs. 19 *c*, and *d*) from a young mosaic leaf of about the same age as the second normal leaf (fig. 19 *b*) showed quite different conditions although they were from the same chlorotic area. In one (fig. 19 *c*) the palisade was very slightly developed and the chloroplasts few, small and very irregular in size. In other sections from the same area (fig 19 *d*), the palisade was almost normal, the chloroplasts almost as abundant as in the section from the normal leaf (fig. 19 *b*) but very irregular in size. A section (fig. 20 *b*) made from the green part of a severely infected leaf showed the same thickness as the section from the normal leaf (fig. 20 *a*) but the chloroplasts were fewer and irregular in size. Three sections made from chlorotic areas of the same diseased leaf (figs. 20 *c*, *d*, *e*) showed that these areas were less than one-half as thick as the normal leaf (fig. 20 *a*) or the green areas of the diseased leaf (fig. 20 *b*) and that the palisade in all cases was under developed. The number of the chloroplasts varied in three sections but they were smaller than in the normal leaf. These studies also show inhibition of the chloroplasts but that the inhibition is overcome with age and exposure to sunlight. They also show that the disease varies in different parts of the same leaf as in the tobacco and tomato which may be due to the age of these areas at time of infection or to severity of infection.

A study of the chloroplasts in a series of leaves from the youngest to the oldest in which the mosaic patterns can be distinguished shows very clearly that the chloroplasts are fewer and smaller in the chlorotic areas than in the green areas of diseased plants or than in the leaves of normal green parts of corresponding ages. They also show that the chloroplasts increase in size and number with age and exposure to sunlight. It should also be noted that there are variations in different areas of the same leaf and in the same area. These variations are apparently dependent on the age of the various areas of the leaves at time of infection or on the severity of these infections or on both. The fact that the leaf is not of a uniform color indicates variations of infection of some kind. The writer is inclined to believe that the active agent spreads unevenly through the leaf and that those areas which are attacked earliest or most severely by the active agent show the greatest amount of inhibition.

These studies on the plastids indicate that in the case of the normal plant and in the green parts of the diseased plants, the chloroplasts are well developed when the leaves open. There is some increase with age but it is not marked. The plastids in the chlorotic areas are few in number and small in size but increase rapidly in number and size with age and exposure to light, and in some cases become almost normal. The writer failed in finding any evidence of disintegration of any kind in either the chloroplasts or cell walls.

Iwanowski (**19**) figures large circles or spheres and the writer has found the same thing in both tobacco and sugar cane. It was impossible to trace any connection between them and the plastids and they appear to be quite different from the spheres described by Sorokin (**32**).

In 1926 Beauverie published a paper (**2**) on the degeneration of chloroplasts, especially in parasitised parts in which he also discussed degeneration as provoked by artificial methods but the author of this paper is unable to determine to what extent his results are comparable to the results described by the students of virus diseases.

The writer found many plastids that appeared to be swollen and either empty or filled with a colorless fluid. They varied in form from spherical to spindle shaped. These were formed in abundance in both diseased and apparently healthy tissues.

Very little has been done in the way of chemical studies on the mosaic diseases of plants but there are two papers that have a bearing on the work of the author. In 1919 Colón (**8**) published a paper on the mosaic disease of the sugar cane in which he said:

"Now, the absorption spectra obtained for the alcoholic (ethyl) solution of chlorophyll from newly yellow-striped young leaves did not in our tests and under the same conditions exhibit any differences from the absorption spectra obtained for the alcoholic (ethyl) solution of healthy young leaves.

* * * * * * *

"Should there have occurred any decomposition of the chlorophyll in the diseased leaves, the absorption spectra obtained form the examination of the alcoholic solution of their chlorophyll would not have, in the first place, been identical with the absorption spectra similarly obtained from healthy leaves. New bands or a modification of the old bands would in all probabilities have been noted, since other cane pigments and many decomposition products of chlorophyll are known to have characteristic absorption spectra. The fact that the decomposition of the chlorophyll would, in the case of the sugar cane, have developed in an acid medium would have defined all the more the changes to be expected since the acid decomposition products of chlorophyll are fairly well known."

In 1928 Dunlap (14) published a paper which he summarized as follows:

"1. The mosaic disease of tobacco reduces the chlorophyll (*a–b*) content below that of normal plants.

"2. A lower-than-normal chlorophyll content is characteristic of mosaic tobacco plants at all stages of growth.

"3. The amount of green pigment in any mosaic leaf of a tobacco plant is below normal.

"4. The clorophyll content of young tissues is somewhat more seriously affected by tobacco mosaic than older tissues.

"5. Older tobacco plants, after infection with mosaic virus, were found to contain subnormal amounts of chlorophyll, especially in the leaves that developed after infection.

"6. The total chlorophyll content of mosaic tobacco plants is seriously reduced."

The results given in these two papers appear to be in harmony with the work of the writer of this paper.

Motile bodies were found in the cells of tobacco, tomato and sugar cane. They were found in the mesophyll, epidermal and hair cells of both diseased and healthy plants. They were never found in the chloroplasts as described by both Eckerson and Sorokin.

SUMMARY

1. This paper is a record of comparative studies on mosaic and healthy plants of sugar cane, canna, tobacco, tomato and cowpea. They deal entirely with the cell structure and cell contents of the chlorotic areas as compared with the green areas of the same plants and with healthy plants. They do not include studies of lesions. The comparisons in every case were made on tissues of corresponding ages.

2. The cell structure of a very young leaf of a normal plant is very uniform, but the differentiation begins very early and is practically complete long before the leaves are full sized.

3. The chlorotic areas of mosaic leaves are thinner than the green areas of the same leaves or than normal leaves of corresponding ages.

4. The active agent inhibits the differentiation of the cell structure from the time that the two come in contact. If the active agent comes in contact with the meristematic tissue at an early period in its development, the structure remains undifferentiated; if at a later period, the tissues remain in the stage of development at time of attack.

5. The spread of the active agent through the leaf appears to be

very uneven, which accounts for the mottling and the variations in development in different parts of the leaf.

6. The reaction of the leaf may depend on the age of the leaf at the time of infection, to the virulence of the virus or to the amount of virus; but the fact that we find variations in both thickness and cell structure in a single leaf and sometimes within a single area indicates that there may be an unequal distribution of the virus throughout the plant or throughout an organ of the plant. This is substantiated by the many records of plants (especially tobacco plants) in which one side only showed the symptoms and of leaves in which the symptoms were more severe on one side than on the other.

7. There is no reason to believe that the cell structure is modified by the virus or active agent. The tissue is inhibited in its development and remains at practically the same stage as when attacked by the virus. There is some growth but it is rarely if ever as rapid as in the green areas or in normal leaves.

8. There is no evidence that the chlorotic area increases in size as a result of the spread of the virus into the green cells adjacent to the chlorotic areas. The chlorotic areas increase in size as a result of cell division and growth of chlorotic areas.

9. The variations in growth of tissues in the bloating of the green areas or pocketing which is so common in mosaic tobacco and to the various deformities in other plants.

10. The development of the plastids is inhibited in both size and number. In the very young, growing leaves, it is difficult to locate them in either fresh or stained material but they develop rapidly with age and exposure to sunlight. The mosaic pattern gradually fades and in many cases cannot be detected in the older leaves.

11. The chloroplasts increase in size and number with age and exposure to sunlight.

12. The author did not find any evidence of disintegration of the chloroplasts.

13. In cases of non-infectious chlorosis, the plants did not become green with age and the chloroplasts did not increase in size and number with age and exposure to sunlight.

LITERATURE

1. **Baur, E.,** Zur Aetiologie der Infektiösen Penachierung. Ber. Deutsch. Bot. Gesell. **22**: 453–460 (1904).
2. **Beauverie, M. J.,** Sur les Modes Degenerescence des Chloroplastes, Particulierement dans le Parasitisme. Comptes Rendus Acad. des Sciences. **183**: 141–143 (1926).

3. **Beijerick**, M. W., Verhandehingen der Köninklijke Akademie van Wetenschappen te Amsterdam. Deel **6**: No. 5. (See also Centb. f. Bakt., Par. etc., II. **5**: 27–33 (1900).
4. **Bouyges, H.,** Sur la Nielle des Feuilles de Tabac. Competes. Rendus **137**: 1303–1305 (1903).
5. **Bremer, G.,** Een Cytologisch onderzock van Strepen-Ziekte bij suikerriet en Andre Planten. Archief. Suik Med.—Ind. Meded. **11**: 337–371 (1926).
6. **Chapman, G. H.,** Mosaic Disease of Tobacco. Mass. Agr. Expt. Station, Bull. No. **175** (1917).
7. **Clinton, G. P.,** Chlorosis of Plants with Special Reference to Calico of Tobacco. Conn. Exp. Sta. Rept. for **1914** (1915).
8. **Colón, E. D.** The Absorption Spectrum of the Chlorophyll in Yellow-Striped Sugar Cane. Journ. of the Dept. of Agri. of Porto Rico. **3**: 43–46 (1919).
9. **Cook, Mel. T.** Histology and Cytology of Sugar Cane Mosaic. Journ. of the Dept. of Agriculture of Porto Rico. **9**: 5–20 (1925).
10. ————————, The Effect of the Mosaic on the Content of to Plant Cell. Journ. of the Dept. of Agric. of Porto Rico. **10**: 229–238 (1926). (Issued in 1927).
11. ————————, Some Effects of Mosaic on the Contents of the Cell. Abst. Phytopathology **15**: 57 (1927).
12. **Dickson, B. T.,** Studies Concerning Mosaic Diseases. MacDonald College. Tech. Bull. No. **2**: 1–108 (1922).
13. **Doolittie, S. P.** The Mosaic Disease of Cucurbits. U.S. Dept. of Agriculture Bull. **879** (1920).
14. **Dunlap, A. A.,** Effects of Mosaic upon the Chlorophyll Content of Tobacco. Phytopathology **18**: 698–700 (1928).
15. **Eckerson, S. H.,** An Organism of Tomato Mosaic. Bot. Gaz. **81**: 204–208 (1926).
16. **Goldstein, B.,** A Cytological Study of the Leaves and Growing Points of Healthy and Mosaic Diseased Tobacco Plants. Bul. Torrey Bot. Club. **53**: 499–600 (1926).
17. **Heintzel, K.,** Contagiose Pflanzenkrankheiten ohne Microben unter besondener Berücksichtingung del Mosaikkrankheit der Tabaksblatter. Inang. Diss. Univ. Erlangen. pp. 1–45 (1900).
18. **Hoggan, I. A.,** A Cytological Studies on Virus Diseases of Solanaceous Plants. Journ. of Agri. Res. **35**: 651–671 (1927).
19. **Iwanowski, D.,** Ueber die Mosaikkrankheit del Tabak-pflanze Zeitschrif. f. Pflansenkh. **13**: 1–40 (1903).
20. **Jagger, I. C.,** A Transmissible Mosaic Disease of Lettuce. Jour. of Agri. Res. **22**: 737–739 (1921).
21. **Jones, P. M.,** Structure and Cultural History of a Mycetozoan Found in Tobacco Plants with Mosaic-Like Symptoms. Bot. Gaz. **81**: 446–459 (1926).
22. **Johnston, J. R.,** The Mosaic Disease of Sugar Cane in 1923. Pamphlet. United Fruit Co., 25 pp.
23. **Koning, C. J.,** Die Fleckenoder Mosaikkrankheit des Hollandschen Tabaks. Zeitschrf. f. Pflanzerkh. **9**: 65–80 (1899).

24. ————————, Del Tabak Etudien uber seine Kultur und Biologie. Amsterdam und Leipzig. p. 71–86 (1900) Reviewed in Centrabt. f. Bakt. **6**: 657 (1900).

25. **Kunkel, L. O.,** A Possible Causative Agent for the Mosaic Disease of Corn. Hawaii S. P. A. Bull. Ser. III, pl. 1. pp. 44–58 (1921).

26. **Matsumoto, T.,** Some Experiment With Azuki-Bean Mosaic. Phyto. **12**: 295–297 (1922).

27. **Mayer, A.,** Ueber die Mosaikkrankheit des Tabaks. Landw. Versuchs-station, **32**: 451–457 (1886) Review in Journ. Mycology, **7**: 382–385 (1894).

28. **Melcers, L. E.,** The Mosaic Disease of the Tomato and Related Plants. Ohio Naturalist **13**: 149–173 (1913).

29. **Rand, F. V.,** Pecan Rosette. Its Histology, Cytology and Relation to Other Chlorotic Diseases. U. S. Bureau of Plant Industry. Bull. **1038** (1922).

30. **Rawlins, T. E.,** and **Johnson, James.,** Cytological Studies of the Mosaic Disease of Tobacco. Amer. Jour. of Bot. **12**: 19–32 (1925).

31. **Smith, F. F.,** Some Cytological and Physiological Studies of Mosaic Diseases and Leaf Variegations. Annals of Missouri Botanical Garden. **13**: 425–484 (1926).

32. **Sorokin H.,** Phenomena Associated with the Destruction of the Chloroplasts in Tomato Mosaic. Phytopathology. **19**: 363–379 (1927).

33. **Westerdijk, Joha.,** Die Mosaikkrankheit der Tomaten. Medeelingen uit het Phytopathologisch Laboratorium. "Willie Commelin Scholten". Amsterdam. Maert. pp. 1–19 (1910).

34. **Wood, A. F.,** The Destruction of Chlorophyll by Oxiding Enzymes. Central f. Bakt., Parasi. u. Infek., II Abt. V. Band. pp. 745–754 (1899).

35. ————————, Inhibiting Action of Oxidase Upon Diastase. Science, N. S., **11**: 17–19 (1900).

36. ————————, Observations on the Mosaic Disease of Tobacco. U. S. Bureau of Plant Industry. Bull. **18,** (1902).

EXPLANATION OF PLATES

PLATE III

Figs. 1 *a, c, e.* Cross sections of normal leaves of sugar cane. *a.* leaf just open; *c.* 3rd. leaf; *e.* 5th leaf. The large cells are sheathing cells which lie next to the fibro vascular bundles. Note that the chloroplasts are normal and practically the same throughout.

Figs. 2 *a, c, e.* Cross section from corresponding leaves from mosaic plant. Note that the chloroplasts are few in number and small but improve with age.

PLATE IV

Figs. 3 *a, c, e.* Longitudinal sections through sheath cells of same as Fig. 1.

Figs. 4 *a, c, e.* Same for mosaic plants.

PLATE V

Figs. 5 *a, c, e.* Section through parenchyma or mesophyll cells. Same as 1 & 3.

Figs. 6 *a, c, e.* Same as 2 & 4.

PLATE VI

Fig. 7 *a.* Canna green part. First leaf rolled. All leaves diseased.

Fig. 7 *b.* Canna chlorotic part. First leaf rolled.

Fig. 8. Canna chlorotic part. Second leaf unrolled.

Fig. 9. Canna chlorotic part. 3rd leaf unrolled.

Fig. 10 *a.* Canna green part. 4th leaf unrolled.

Fig. 10 *b.* Canna green part. 4th leaf unrolled.

PLATE VII

Fig. 11 *a.* Tobacco. Normal leaf in bud. Slightly younger than 11*b*.

Fig. 11 *b.* Tobacco. Mosaic leaf in bud. Note that the cell structure of *a* and *b* are practically the same. Also that there are plastids in 11 *a* and none in 11 *b*.

Fig. 12 *a.* Tobacco. Normal leaf. About 2 inches in length.

Fig. 12 *b.* Tobacco. Mosaic leaf. Less than 2 inches in length.

Figs. 12 *a* & *b.* Same age. Note the difference in thickness and that 12 *a* has the palisade tissue. Also the difference in the chloroplasts.

PLATE VIII

Fig. 13 *a.* Tobacco. Normal leaf. About 3½ inches; rather thin.

Fig. 13 *b.* Tobacco. Mosaic leaf. About 3½ inches. Note that these two leaves are practically the same except that development of the chlorophyll is better in *a* than in *b*.

Fig. 13 *c.* Tobacco. Mosaic leaf. Same age as 13 *b*. The infection more severe and palisade poorly developed.

PLATE IX

Fig. 14 *a.* Tobacco. Normal leaf. About 6 & 8 inches.

Figs. 14 *b* & *c.* Tobacco. Mosaic leaf. About 6 & 8 inches. The disease in both *b* and *c* is mild; the palisade is well developed but the chloroplasts reduced.

Plate X

Fig. 15 *a*. Tomato leaf. Green part. Youngest leaf.

Fig. 15 *b*. Tomato leaf. Chlorotic part. Youngest leaf. Note the difference in cell structure and chloroplasts.

Fig. 16 *a*. Tomato leaf. Green part. 2nd leaf.

Figs. 16 *b* & *c*. Tomato leaf. Chlorotic part. 2nd. leaf.

Figs. *b* and *c*. From same section of leaflet. One on one side of midrib and one on other. Note difference in structure and chloroplasts. Also compare with 16 *a* and with 15 *a* and *b*.

Plate XI

Fig. 17 *a*. Tomato leaf. Green part. 3 leaf.

Fig. 17 *b*. Tomato leaf. Cholorotic part. 3 leaf. This leaf was attacked later than 16 *a*. The structure is slightly reduced and the chloroplasts decidedly reduced.

Fig. 18 *a*. Tomato leaf. Green part. 4 leaf.

Figs. 18 *b*, *c*. Tomato leaf. Chlorotic part. 4 leaf.

Fig. *b* was attacked early and *c* late. Note difference in structure. Compare chloroplasts with 18 *a*.

Plate XII

Fig. 19 *a*. Cowpea. Very young leaf. Normal.

Fig. 19 *b*. Cowpea. Young leaf. Normal.

Fig. 19 *c*. Cowpea. Young leaf. Mosaic.

Fig. 19 *d*. Cowpea. Same leaf. Mosaic.

Compare structure and chloroplasts; *c* and *d* are from same leaf which shows that the disease was more severe in one part than in the other.

Plate XIII

Fig. 20 *a*. Cowpea. Old leaf. Normal.

Fig. 20 *b*. Cowpea. Old leaf. Mosaic. Green part. Note the double layer of palisade and compare chloroplasts. The disease in this case was very mild.

Plate XIV

Fig. 20 *c*. Cowpea. Old leaf. Mosaic. Severe.

Fig. 20 *d*. Cowpea. Old leaf. Mosaic.

Fig. 20 *e*. Cowpea. Old leaf. Mosaic.

Note that the cell structure in *c*, *d* & *e* is abnormal but that the chloroplasts tend to recover.

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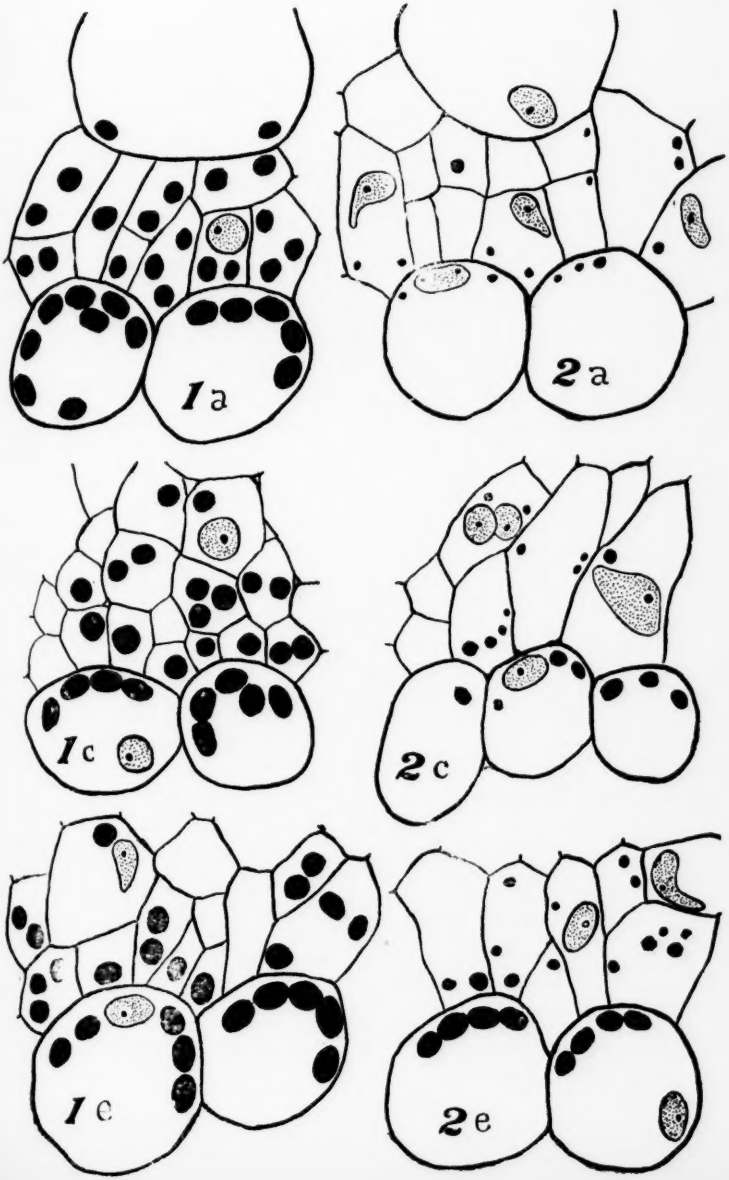
PLATE III



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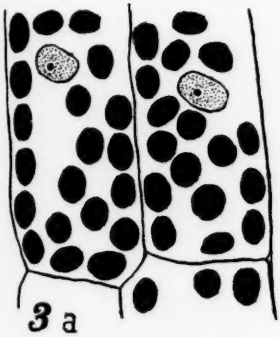

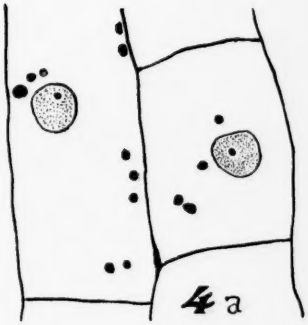

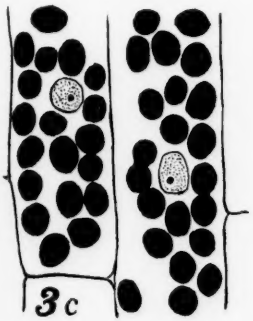

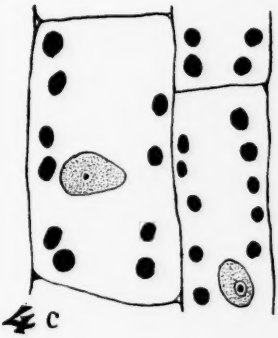

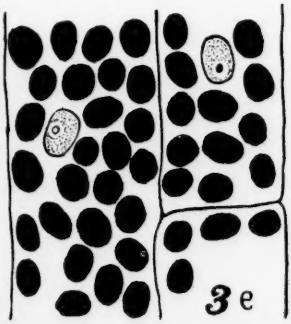

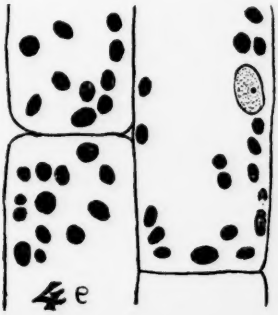

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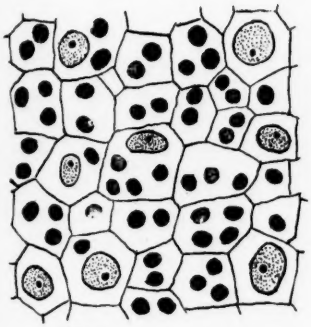

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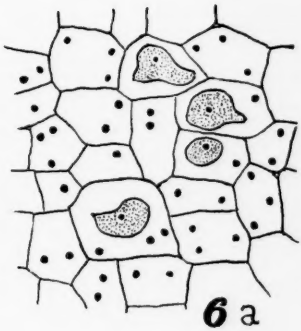

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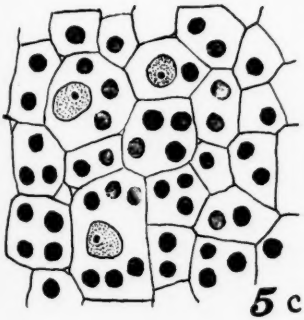

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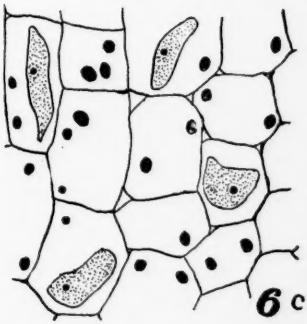

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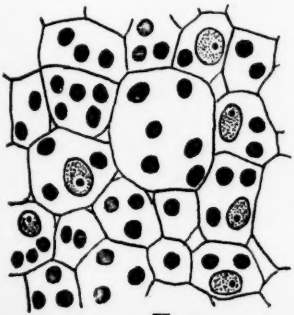

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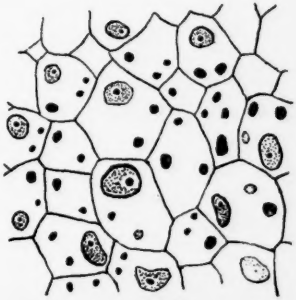

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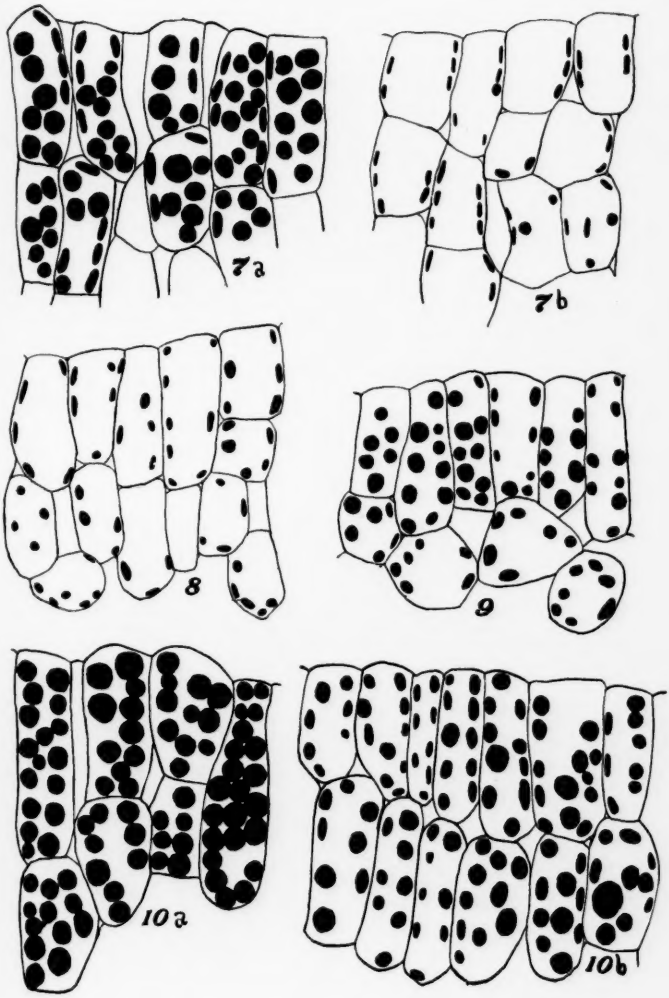

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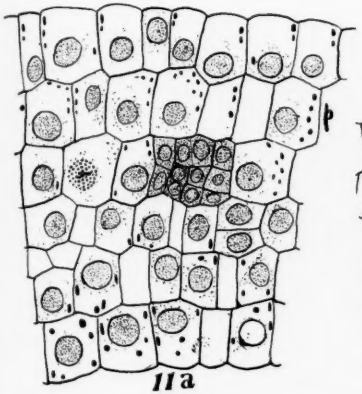

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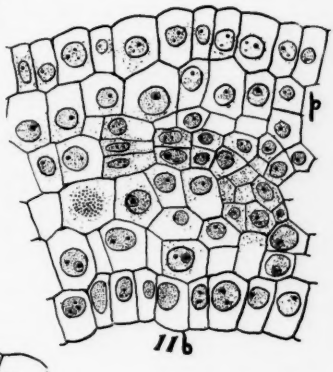

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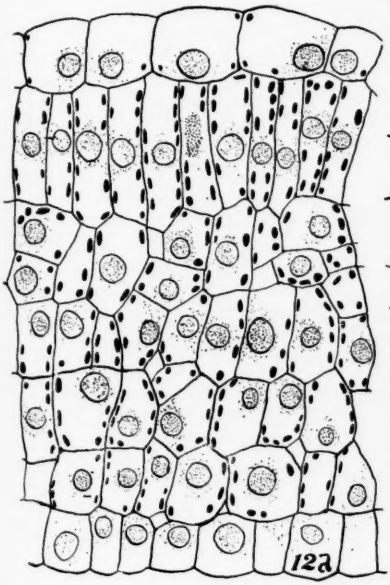

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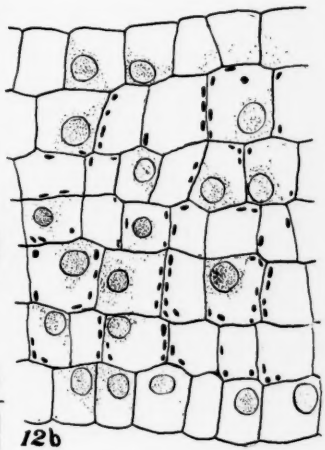

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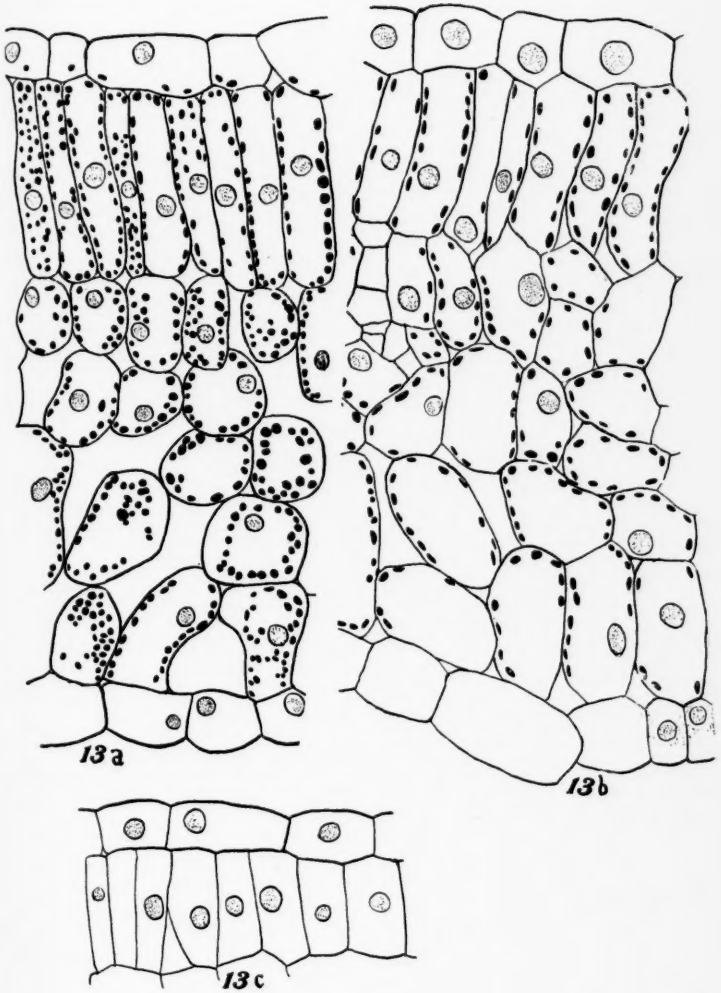

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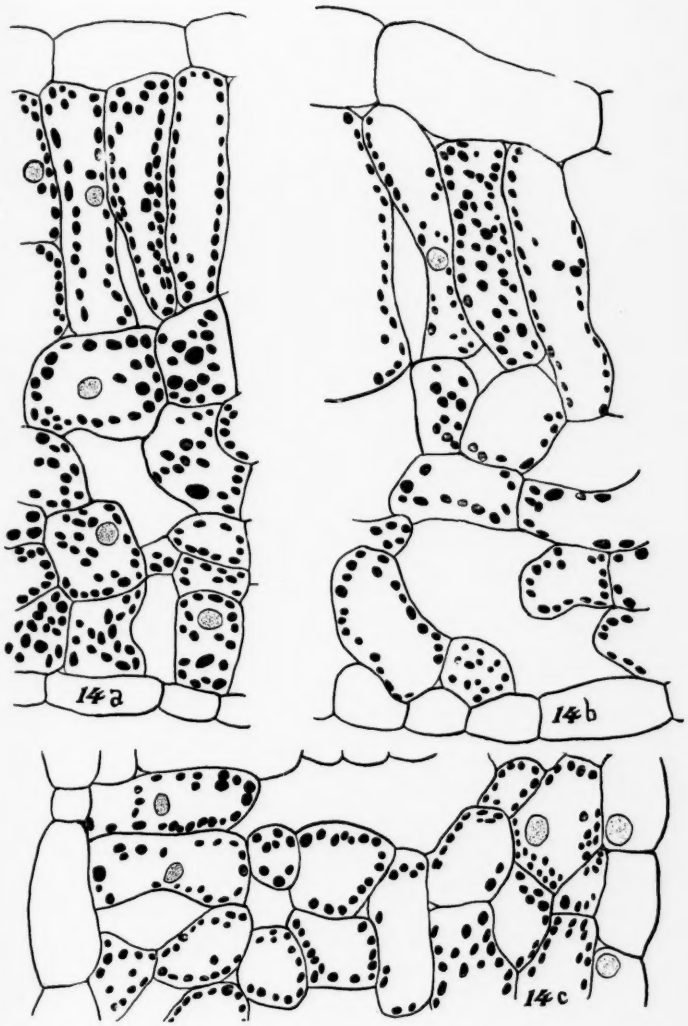

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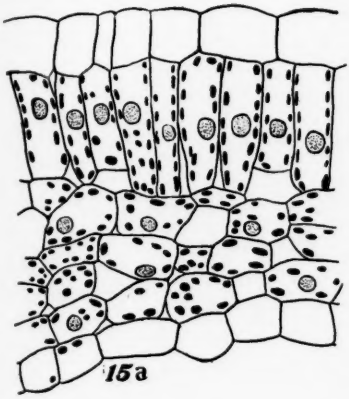

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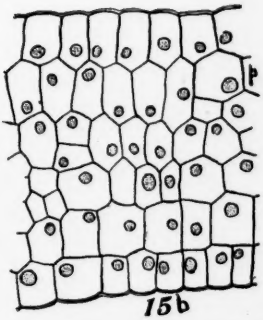

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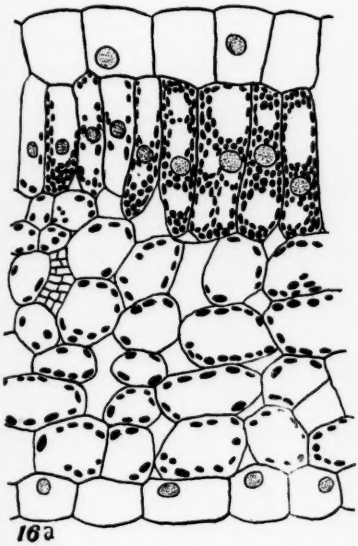

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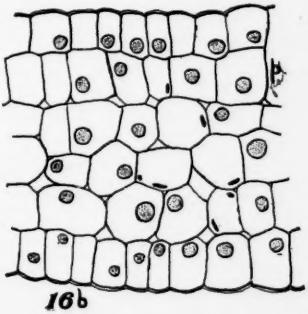

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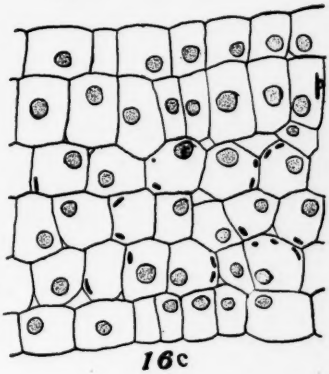

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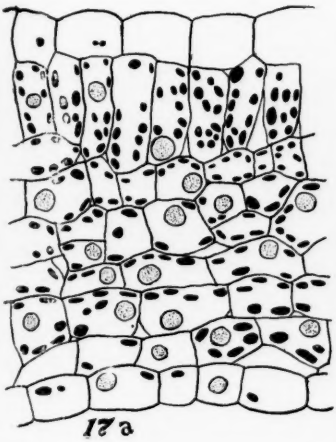

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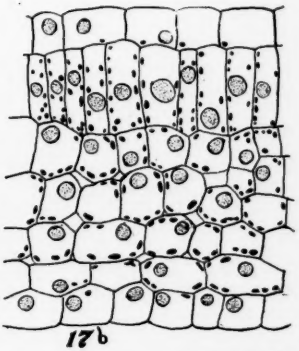

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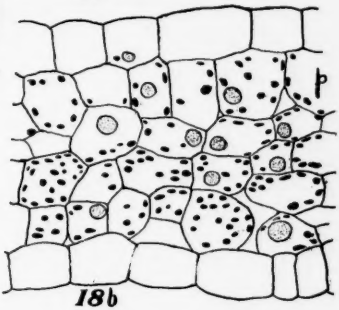

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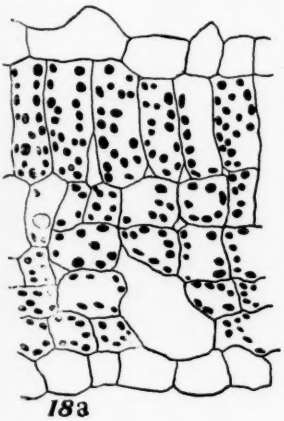

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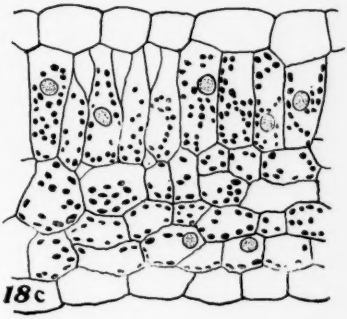

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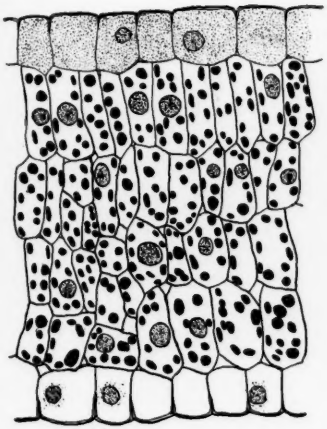

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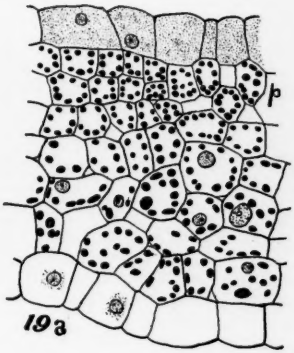

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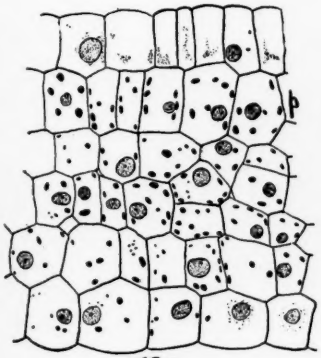

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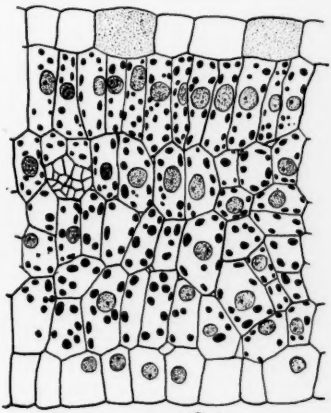

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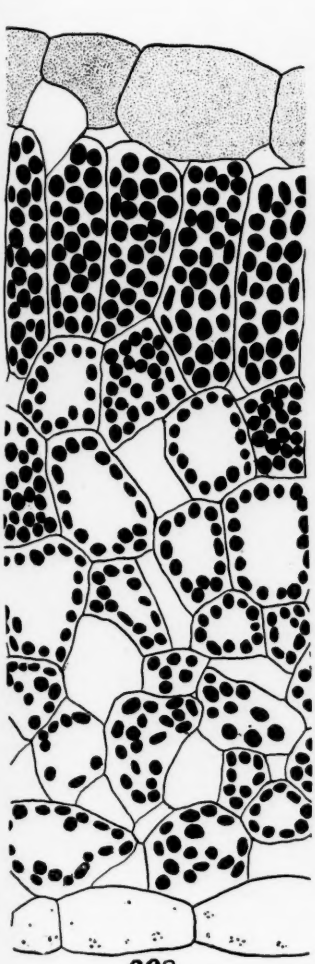

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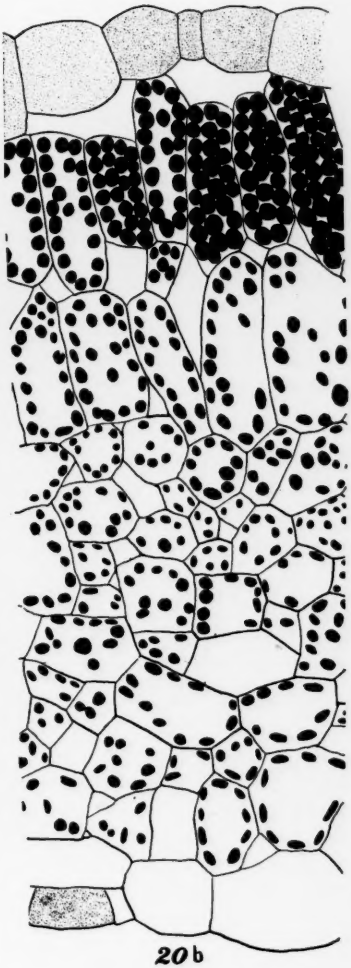

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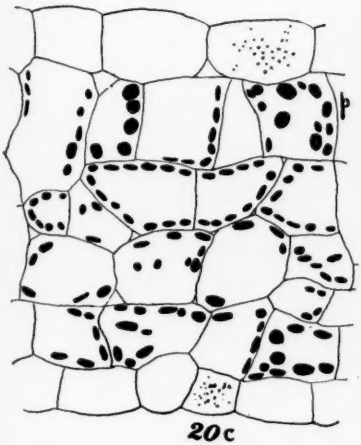

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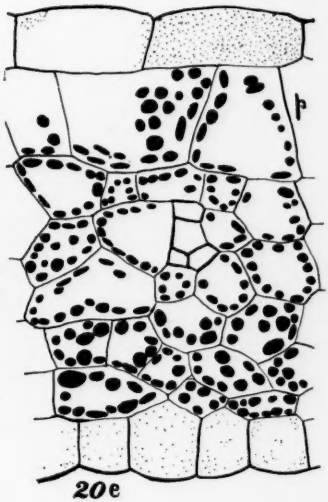

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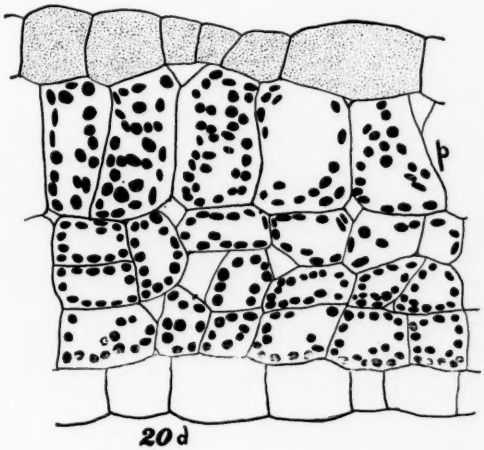

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EDITOR.